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Model Organisms to Models: How Ageing Affects Infectious Disease Dynamics

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A thesis submitted for the degree of Doctor of Philosophy

The University of Edinburgh

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Declaration

I declare that this thesis has been composed by myself and that the work has not been submitted for any other degree or professional qualification. I confirm that the work submitted is my own, except where work which has formed part of jointly-authored publications has been included. My contribution and those of the other authors to this work have been explicitly indicated below. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others.

The work presented in Chapter 2 was previously published in *Ecology Letters* as **Disease Spread in Age Structured Populations with Maternal Age Effects** by Jessica Clark (Author of Declaration & Student), Jenny Garbutt (Research Group Member), Luke McNally (Collaborator) & Tom Little (Primary Supervisor). This study was conceived by all of the authors. I wrote the manuscript with comments on previous versions by LM and TL. I carried out all data analysis. I developed and implemented the mathematical model. Jenny Garbutt collected all experimental data.

The work presented in Chapter 3 has been submitted to *PNAS* as **Pathogen Dynamics Across the Diversity of Ageing** by Jessica Clark (Author of Declaration & Student), Luke McNally (Collaborator) & Tom Little (Primary Supervisor). This study was conceived by all of the authors. I wrote the manuscript with comments on previous versions by LM and TL. I collected all experimental data apart from that used in the mortality analysis. I carried out all data analysis. I developed and implemented the mathematical model with guidance from Luke McNally (Collaborator).

In Chapter 4, Experiment 1 was conceived by Tom Little (Primary Supervisor) and the data was collected by Phil Wilson. Experiment 2 was conceived during a conversation between Tom Little (Primary Supervisor) and me. Experiment 3 was conceived during a conversation between Tom Little (Primary Supervisor) and me. Unless otherwise stated I collected the data for this chapter. I analysed all data for this chapter. I am the author of this chapter. Tom Little (Primary Supervisor) provided comments on previous versions of the chapter.

The experiments in Chapter 5 were conceived during a conversation between me, Dr. Pedro Vale and Tom Little (Primary Supervisor). I collected and analysed all data. I am the author of this chapter. Tom Little (Primary Supervisor) provided comments on previous versions.

I was assisted in the development of Chapter 6 by Luke McNally (Collaborator). I am the author of this chapter with comments on previous versions from Tom Little (Primary Supervisor).

A handwritten signature in black ink, reading "Jessica Clark". The signature is written in a cursive, flowing style. The first name "Jessica" is written in a more compact, rounded script, while the last name "Clark" is written in a more open, elongated script with a prominent loop at the end.

Jessica Clark. January 2019.

Lay Summary

Populations are often made up of individuals of different ages. These individuals of different ages often have different reproductive capabilities, risks of dying and susceptibilities to infection. Reproduction, mortality and susceptibility are also important aspects of infectious disease dynamics. Despite these parallels, and how common it is for populations to be age-structured, little research has explored how characteristics of population age structure impact disease spread. The aims of this thesis were therefore to investigate how host age, maternal age and population age structure influence infectious disease dynamics and pathogen evolution. Using the freshwater crustacean *Daphnia magna*, I carried out experiments, and show that old individuals produce fewer offspring with age, but that offspring from these old mothers perform equally or better than offspring from young mothers across a suite of traits, in particular, they are more resistant to infection. These old mothers also do not produce offspring that may cause more infections than the population average. Alternatively, young hosts are potentially more infectious than old hosts. I also show that there is a lot of genetic variation in overall physiological performance in *Daphnia* and explore what maintains this variation. Drawing on these experimental observations I develop a series of mathematical models to show that age-specific susceptibility, maternal effects on offspring susceptibility, age-specific rates of reproduction and age-specific rates of mortality, can all affect infectious disease dynamics. I also show that these dynamics are dependent on ecological conditions. In light of rapidly ageing global populations I also show that changes in global population age structure can cause changes to how much damage a pathogen causes to its host.

Abstract

Population age structure is a ubiquitous population characteristic, with individuals of different ages in a population often displaying different physiological capabilities, like rates of reproduction and mortality, or different immune capabilities. These measures of performance are also key parameters in epidemiology, however, how heterogeneities in age-specific performance influence pathogen dynamics has received little attention. Investigating the impact of ageing on pathogen dynamics is hampered by a lack of information on whole organism age-specific performance beyond fecundity and mortality rates, particularly outside mammals. It was therefore the ambition of this thesis to integrate experimental observations of individual ageing patterns with mathematical epidemiological models to understand how individual ageing may influence population level pathogen dynamics. This thesis is comprised of a series of experiments using the invertebrate *Daphnia magna* and bacterial pathogen *Pasteuria ramosa*, and a series of models informed by the observed biological results. My experimental results reveal that ageing is asynchronous across traits within a single organism, and that ageing should be considered across generations: fitness measured only as offspring quantity may not be synonymous with fitness measured as a product of offspring quality. I go on to show that there is considerable genetic variation in age-specific performance and longevity in *D. magna*, but there was no evidence to suggest that this is maintained through genetic trade-offs. Furthermore, despite this genetic variation in age specific performance, young hosts always carry higher pathogen loads, and as such, age should be considered as more than just contact rates in the quest to determine what makes a host a super-spreader. The epidemiological models developed in

this thesis, show that ageing effects on host susceptibility within and between generations have a direct effect on transmission events, whilst ageing effects on fecundity and mortality rates indirectly drive pathogen dynamics via effects on demography and population density. These drivers of pathogen dynamics also may cause an increase in pathogen transmission if a successful intervention to human ageing is applied. The changes to these vital parameters of epidemiology associated with ageing populations can also result in changes to the optimal level of pathogen virulence. The data presented in this thesis provides novel insight into the many ways in which host age, maternal age and population age structure may influence pathogen dynamics. Much of this work challenges longstanding dogma of ageing theory and is highly relevant in light of ageing human populations, thus providing a tool for evolutionary biologists, demographers and epidemiologists alike.

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Chapter 1 General Introduction

Background

Parasites are pervasive across taxa having evolved many diverse host exploitation strategies (Sheldon & Verhulst, 1996, Dobson et al., 2008). The prevention and control of the ever-growing number of outbreaks is an ongoing drain on global resources (Smith et al., 2012, Smith et al., 2014). As the interactions between hosts and their parasites evolve, an understanding of evolutionary processes to meet present and future challenges of infectious diseases is required (Little et al., 2012).

Heterogeneity in host condition within populations is commonplace, shaping infection dynamics by determining an individual's susceptibility or how well a pathogen grows within a host (Beldomenico & Begon, 2010, Krist et al., 2004, VanderWaal & Ezenwa, 2016a, Tschirren et al., 2007, Tseng & Myers, 2014). Common sources of heterogeneity in host condition are genetics and age. The genetics of host-parasite interactions have received considerable attention (Little & Ebert, 1999, Staskawicz et al., 1995, Fellous et al., 2012, Blandin et al., 2009, Howick & Lazzaro, 2014, Little, 2002, Decaestecker et al., 2007, Decaestecker et al., 2003), but may be only a small part of the prevalence and intensity of natural infections (Krist et al., 2004).

Comparatively, the role of host age, characteristics of ageing, or population age structure, has received little attention in the study of in pathogen dynamics (Izhar & Ben-Ami, 2015, Worby et al., 2015, Wallinga et al., 2006, Andreasen, 1989, Clark et al., 2017, Izhar et al., 2015). Pathogen dynamics are a function of host reproduction rates, mortality rates and susceptibility to infection (Anderson & May, 1979). Performance in these host characteristics

often changes with age, though comprehensive investigations into whole organism ageing, particularly beyond fecundity and mortality, are uncommon. For example, offspring condition is also an important component of reproduction, with evidence suggesting maternal condition influences offspring performance (Boots & Roberts, 2012, Garbutt & Little, 2017, Garbutt et al., 2014b, Kaneko et al., 2011, Mitchell & Read, 2005). Without these considerations, investigations into the role of ageing on pathogen dynamics are limited.

In this thesis, I therefore aim to investigate the effects of host age, maternal age, and population age structure, on pathogen dynamics. To address these aims required an in-depth characterisation of the differences in physiological capability and performance between individuals of different ages. Though I draw on ageing theory and literature it was not the aim of this thesis to exhaustively test the predictions of ageing theory. To characterise individual age-specific differences in physiological performance I conducted experiments on the highly tractable laboratory model system *Daphnia magna*, using a natural bacterial parasite of *D. magna*, *Pasteuria ramosa* for infections. I also developed a number of epidemiological models to investigate how these observed differences, or wider variation, in age-specific performance may influence pathogen dynamics and pathogen evolution in age structured populations.

Longevity, Host Age & Pathogen Dynamics

Ageing Theory

An individual's age is assumed to be synonymous with their health and vitality. Those who are young are expected to be in their physiological

prime, with physiological capability invariably declining with an increase in age. Preceding the end of an individual's lifespan, senescence is characterised by an increasing probability of mortality and a decline in reproduction. The lack of obvious fitness gains attributed to this period of decline has generated extensive discussion as to how senescence and limitations to longevity evolved.

Weismann (1889) initially proposed that senescence evolved because organisms that split germ and soma must invest more heavily in reproduction than soma resulting in physiological decline. Kirkwood (1977) built upon this concept in more detail with the Disposable Soma theory. He theorised that an accumulation of mutations later in life occurs due to the reallocation of energy from proof reading and other accuracy devices to reproduction and development, resulting in observable senescence - thus trading off physiological health and longevity with overall reproductive success (Gavrilov & Gavrilova, 2002, Kirkwood & Holliday, 1979, Monaghan, 2008).

An alternative, and also widely supported theory, is that senescence is caused by the weakening of selection with an increase in age. Once an individual has reproduced, deleterious alleles have been passed on to offspring, and so selection is inefficient at removing them from a population (Haldane, 1941, Charlesworth, 2000, Hamilton, 1966), resulting in a selection shadow (Fabian & Flatt, 2011, Flatt & Partridge, 2018).

Recognising this point, Medawar (1952) proposed that alleles beneficial early in life are favoured over alleles beneficial later in life (i.e. post onset of reproduction), so that deleterious alleles accumulate passively across successive generations, resulting in increased age associated mortality and

the observable physiological decline associated with age (Gavrilov & Gavrilova, 2002, Medawar, 1952). Also recognising the weakening of selection with age, Williams (1957) and Rose (1984a) developed the Antagonistic Pleiotropy (AP) theory of ageing – proposing that alleles acting positively in early life, even if negative in late life, are actively selected to remain within the population. Genetic trade-offs indicative of AP can be detected as phenotypic life-history trade-offs for example between early and late life reproduction or sexual and non-sexual traits, such as reproduction and immune investment (Arbuthnott, 2018). Genetic variation in life history traits is generally proposed to be maintained through such mechanisms, resulting in observable trade-offs indicative of genotype-by-environment interactions.

Despite the longstanding prediction that senescence is an inevitable outcome of ageing, there is evidence to suggest it is not that simple. Instead, the trajectories of ageing in measures like fecundity and mortality occur on a spectrum from juvenescence where some performance measures can improve with age, to senescence where others may decline with age as predicted by theory (Jones et al., 2014). There is however, more to ageing than fecundity and mortality, for example, it is common for immune capability to change with age in many organisms (Garbutt et al., 2014a, Simon et al., 2015). Work in *Drosophila* and mice has shown that the application of dietary restriction – that is the modest reduction of dietary intake above malnutrition – results in a reduction or delay in immune gene expression in exchange for increased longevity (Lee et al., 1999, DeVeale et al., 2004, Pletcher et al., 2002, Seroude et al., 2002). This suggests that the immune system supports early life survival and therefore fitness gains (DeVeale et al., 2004), but at the expense of lifespan.

Host Longevity & Pathogen Dynamics

Host longevity is an important selective force in pathogen dynamics. Those with short life spans or undergoing senescence have a higher intrinsic rate of mortality than long-lived or young hosts limiting the duration of infection and therefore pathogen fitness. It has been theoretically shown that it is these hosts with higher intrinsic mortality that may experience more aggressive infections, as parasites maximise their reproduction to maintain transmission, in light of a decreased duration of infection (Carlsson-Granér & Thrall, 2006). Alternatively, aggressive infections in long-lived hosts are thought to decrease pathogen persistence, such that parasites adopt a “milker-like” strategy with low replication in populations with long lifespans (Viljoen et al., 2018). The force of host mortality is also thought to influence host evolution by influencing costly investment in resistance. It is generally considered that long-lived hosts will invest more in resistance than short-lived hosts (Carlsson-Granér & Thrall, 2006). The nuances and exceptions of this are discussed in Donnelly, White & Boots (2017), where mixed infections, and generalist vs. specific immune responses are considered.

Age Specific Physiology

Age specific responses to pathogen challenges are common across taxa. It has long been considered that invertebrates have only innate immune systems, that are hardwired and uniform in response to new pathogenic challenges. By comparison, vertebrates have both innate and an acquired immune system, that develops further with age and in response to novel challenges, providing protection from future reinfection. It is therefore considered the norm in vertebrate systems to see an increase to a peak in pathogen defence capability with age, which then wains with age as

immuno-senescence sets in (Simon et al., 2015). The notion of this taxonomic split has more recently been challenged however (Rimer et al., 2014, Little et al., 2005) as evidence from invertebrates like *Daphnia magna*, the honey bee *Apis mellifera*, the American Cockroach *Periplaneta americana*, *Drosophila* and the house cricket *Acheta domesticus* (Lesser et al., 2006, Piñera et al., 2013, Rheins & Karp, 1985, Garbutt et al., 2014a, Wilson-Rich et al., 2008) suggest a process of pathogen defence development in invertebrates also.

Further to the direct effects of age on immune capabilities, age also influences key epidemiological parameters such as mortality rates and reproduction. As a female gets older the number of offspring produced may change (Jones et al., 2014, Hayward et al., 2015). This may indirectly affect pathogen dynamics by altering the supply and density of susceptible hosts in a population. There is also more to reproductive ageing than the quantity of offspring a female produces, as the quality of these offspring, which most would assume to decline, is also an important factor to consider.

Host Maternal Age & Pathogen Dynamics

In contrast to changes in phenotype due to DNA sequence changes associated with heritable recombination, maternal effects are the non-genetic reflection of the maternal environment in the offspring phenotype. This results in natural selection acting on phenotypic variation that is caused by events in the previous generation (Mousseau, 1991). There are many ways in which mothers can influence their offspring including offspring provisioning, mate choice and offspring development (like the production of resting eggs in response to environmental cues (Deng, 1996)) (Mousseau & Fox, 1998). Maternal effects can influence offspring resistance to infection,

increasing offspring resistance when mothers experience poor environments such as increased temperatures (Garbutt et al., 2014b) or poor nutrition availability (Boots & Roberts, 2012, Mitchell & Read, 2005). The infection status of a female can also have an effect on offspring performance in the face of pathogen challenge. The archetypal example is that of vertebrates where mothers transfer antibodies to their offspring providing immune support during early life (Hasselquist & Nilsson, 2009). Highlighting just how little we know of invertebrate immune systems, it has been shown that this transfer of immunity, long thought to be an ability of vertebrates, can also occur between invertebrate mothers and offspring (Little et al., 2003).

Maternal effects may also impact offspring negatively. For example, according to the Lansing Effect, maternal effects as a result of increased maternal age can negatively impact offspring reproduction, longevity and the rate of offspring senescence (Lansing, 1947). This is in keeping with observations from humans, where congenital disorders are seen more commonly in offspring of older mothers (Kenny et al., 2013) or with other taxa, where negative impacts of advancing parental age on offspring life-history have been recorded (Comfort, 1953, Hjelmborg et al., 2015, Benton et al., 2005, Plaistow et al., 2015b, Benton et al., 2008). Evidence suggests however, that maternal effects are organism specific, as an increase in maternal age may also result in larger offspring, particularly in fish and invertebrates with indeterminate growth. An increase in body size can imply offspring of better quality (see Marshall et al., 2010 & references within) and has been linked previously with pathogen resistance (Garbutt & Little, 2017). The possible variation in phenotypic traits, as a response to the maternal environment, suggests maternal age effects could therefore drive population

and epidemiological dynamics (Beckerman et al., 2002, Benton et al., 2008, Gaillard et al., 2003)

Population Age Structure & Pathogen Dynamics

The ecology of many diseases, such as helminth infections and childhood viruses, are inherently age structured (Heininger & Seward, 2006, Anderson & May, 1985, Anderson & Medley, 1985, Zhao et al., 2016, Otsu et al., 2011). An infectious agent that elicits life-long future resistance to reinfection, or concomitant immunity, increases the probability that old hosts are resistant to reinfection (Andreasen, 1989, Laskowski et al., 2011, Chan et al., 1995, Chan et al., 1994, Anderson & May, 1985, Woolhouse et al., 1991). However, there are further parallels between population age structure and epidemiology, beyond age-specific immune function, that have gone largely unconsidered in an epidemiological context. For example, population age structure is the product of reproduction and mortality rates (Goldstein, 2009), which, in addition to age-specific susceptibility, are also key epidemiological parameters. Reduced reproductive rates and increasing mortality rates are indicative of ageing populations (Goldstein, 2009). It is therefore conceivable that the currently occurring rapid ageing of human populations (He et al., 2016), could have epidemiological consequences (Kline & Bowdish, 2016).

Population structure in its many forms can be an architect of pathogen dynamics. For example, modelling of influenza virus transmission in age structured populations has shown that depending on population density, the population age distribution can play a significant role in the transmission of influenza (Laskowski et al., 2011). Other forms of population structure have

received considerably more attention than age structure, particularly in the context of virulence evolution. For example, a general rule of thumb in population contact and spatial structure literature is that lower levels of virulence will occur in populations with restricted contact structures, but higher levels in more connected populations (Cressler et al., 2016). Though largely rooted in theory, there is empirical support for these predictions. Work on a nematode-wasp system has shown that population structures supporting more opportunities for pathogen transmission see more virulent pathogens (Herre, 1993). Experiments with a moth larvae – virus system have also shown that population viscosity determines the level of virulence the virus evolves towards (Boots & Meador, 2007). Further to spatial structure, population sex ratio has also been considered with theory predicting the evolution of sex-specific virulence strategies in populations with sex-specific heterogeneities in transmission routes (Ubeda & Jansen, 2016). Heterogeneities in a population for factors linked to infectious disease dynamics, like susceptibility to infection, and tolerance can also exacerbate the effects of co-occurring population structure on virulence evolution (Cousineau & Alizon, 2014). It is therefore apparent that population structure can influence pathogen dynamics. As age is a common source of host heterogeneity, the role of population age structure, clearly requires further investigation.

Model Systems to Modelling and Back Again

Daphnia magna

The experimental work in this thesis was conducted using *Daphnia magna* (order: Cladocera), a zooplanktonic freshwater crustacean that is cyclically parthenogenic, producing either diploid subitaneous eggs or resting

(recombinant) eggs. The initiation of sexual reproduction is environmentally driven and often varies by genotype (Deng, 1996, Kleiven et al., 1992). The experiments in this thesis used only clonally reproduced offspring, controlling for genetic differences between hosts, allowing the exploration of these genetically derived differences when applicable. Parthenogenically reproducing females deposit eggs into the brood chamber after every adult moult (Anderson & Jenkins, 1942), where they develop until they are ejected, resulting in offspring about every three days. Females will continue to reproduce until they die. In this thesis 60 days of age was considered “old” whereby after this point a significant increase in mortality was observed, however, survival was recorded up to 160 days in some instances (Chapter 4) with reproduction continuing to occur. This results in a highly tractable system where reproduction rates and dates and mortality as a function of age or maternal age are easily recorded over two generations within a matter of weeks or months depending on the duration of observation. This made *D. magna* an ideal system for experiments on longevity, ageing, and reproduction.

Pasteuria ramosa

Infections were carried out using *Pasteuria ramosa* (Metchnikoff 1888), a spore forming natural bacterial parasite of *D. magna*. *Pasteuria ramosa* is a gram-positive obligately killing bacteria, requiring host mortality for environmental transmission. The host will come into contact with dormant transmission stage spores whilst filter feeding particles suspended in the water column or by foraging in sediment where spores comes to rest, and where they can remain for a considerable length of time (Decaestecker et al., 2007). It is believed the spores then penetrate the oesophagus to enter the haemolymph of the host (Ebert et al., 2016), causing castration within 5-

15 days of infection and gigantism (Ebert, 2005). Infected *D. magna* are easily identified as the bacterial growth fills the transparent host with a reddish-brown mass, obscuring the transparency. Further microscopic investigation will also reveal spores within the host that vary in developmental stage, particularly dependent on the length and stage of infection (see Ebert et al., 2016 for a full characterisation of the infection process and identifiable spore morphology). As the pathogen remains in the host from infection until death, and recovery is rare (Clerc et al., 2015), it is possible to easily quantify the duration of infection and pathogen load. Furthermore, owing to the tractability of *D. magna* life history, it is possible to delineate the effects of genetics and host condition (as a result of age, or food or other environmental variables (Vale et al., 2013, Vale et al., 2011)) on pathogen dynamics.

Epidemiological models

Epidemiological modelling is a pillar of public health policy, opening up the potential to probe non-linear and potentially intractable infectious disease dynamics. Models can be classified dependent on the assumptions that underlie them and the types of dynamics they attempt to explain. For example, deterministic vs. stochastic models differ in how they handle parameter uncertainty and variability, while continuous or discrete models handle time in different manners. Models may also be spatially structured, or consider only homogenous or heterogeneous mixing patterns between hosts (Garner & Hamilton, 2011). The range of scenarios epidemiological models can be applied to has consequently led to their successful use in the development of mitigation strategies like vaccination programmes, (Anderson & May, 1982b, Meltzer et al., 1999, Müller, 2000, Ramsay et al., 1993), the prediction of emerging diseases (Brierley et al., 2016) and the

generation of hypotheses surrounding the factors contributing to infectious disease spread (Lloyd-Smith et al., 2005).

A central component of many of these models is the basic reproductive ratio R_0 . Heesterbeek (2002) gives a thorough overview of the history of R_0 , which originated in demographic modelling, and with which it still shares many similarities despite being applied to different questions. In epidemiology, R_0 is defined as the total number of secondary infections that arise from the introduction of a novel pathogen into a fully susceptible population. It is a unitless metric that is dependent on the probability of infection dependent on how susceptible the uninfected hosts are, the average rate of contact between infected and uninfected individuals, and the duration of infectiousness which is a product of natural host mortality rates, clearance of the pathogen, and pathogen induced mortality rates. This value can then be used to determine the epidemic capability of a pathogen. If $R_0 > 1$ then a pathogen will spread, and if < 1 it will die out. In addition to determining epidemic potential, pathogen R_0 can also be used to elucidate elements of pathogen evolution (Frank, 1992).

The models in Chapters 2 and 3, are systems of dynamic equations that are used to derive pathogen R_0 . They were developed based on experimental observations and were therefore made with biologically inferred assumptions. The model in Chapter 3 extends these observations to consider more broadly, patterns of ageing recorded across taxa. The model in Chapter 6 considers qualitative pathogen characteristics that can be derived from pathogen, R_0 in a framework applicable to many pathogens. Coupling controlled experimental studies with epidemiological modelling,

allows accurate model development and the use of valid assumptions, in addition to providing a way to test model results in the future. In turn, the production of data, derived from model hypotheses can be used to develop and parameterise future models, thus making the integration of model systems and epidemiological modelling a valuable tool in epidemiological research.

Thesis outline and aims

This thesis combines empirical investigations with mathematical modelling to investigate the effects of host age, maternal age, and population age structure, on pathogen dynamics and evolution.

In Chapter 2 I show that old *D. magna* mothers produce offspring of equal or better quality, but in particular, they produce offspring that are more resistant to infection from *P. ramosa* than offspring from younger mothers. Considering this alongside previous observations that old *D. magna* are also more resistant to infection. I then developed a compartmental Susceptible – Infected model to show that this biologically realistic ageing profile benefits pathogen transmission.

In Chapter 3 I compile a whole organism ageing profile by experimentally characterising ageing across two generations of *D. magna*, considering offspring performance as a function of maternal age, as an under-appreciated aspect of reproductive ageing. I then develop a compartmental Susceptible – Infected model, that explicitly allows for ageing in 4 traits believed to be important in pathogen transmission. With this model I show that host ageing patterns are an important component of infectious disease

dynamics. I also show how intervention in human ageing may have an affect pathogen transmission.

In Chapter 4 I investigate between and within population genetic variation and its maintenance in life history traits relevant to work throughout this thesis, due to their connection to pathogen dynamics. I show that, contrary to common intuition, there is as much genetic variation within a population as between populations in key life history traits. I also show no evidence of genetic trade-offs, indicative of Antagonistic Pleiotropy in the *D. magna* – *P. ramosa* system.

In Chapter 5, I experimentally investigate how host age and maternal age affect pathogen growth and transmission potential in the context of super-spreading using *D. magna* and *P. ramosa*. I show that young hosts harbour higher pathogen loads with greater aggregation and dispersion than old hosts, whilst maternal age does not influence pathogen distribution.

In Chapter 6, I develop a model for the evolution of pathogen optimal virulence, that explicitly considers age specific susceptibility to infection, clearance rates, intrinsic mortality rates and transmission. I then parameterise the model using publicly available demographic and infection data. With this model I show that changes in global population age structure between 2016 and 2050 may impact future pathogen optimal virulence in a diverse group of infectious diseases of global concern.

Chapter 2 Disease Spread in Age Structured Populations with Maternal Age Effects

Summary

Fundamental ecological processes such as extrinsic mortality determine population age structure. This influences disease spread when individuals of different ages differ in susceptibility, or when maternal age determines offspring susceptibility. We show that *Daphnia magna* offspring born to young mothers are more susceptible to *Pasteuria ramosa* than those born to older mothers and consider this alongside previous observations that susceptibility declines with age in this system. Thus, we used a Susceptible-Infected compartmental model to investigate how age-specific susceptibility and maternal age effects on offspring susceptibility interact with demographic factors affecting disease spread. Our results show a scenario where an increase in extrinsic mortality drives an increase in transmission potential. Furthermore, we identify a realistic context in which age effects and maternal effects produce conditions favouring disease transmission.

Introduction

The ecological context of a host species will shape key demographic features, which in turn affect disease spread. Extrinsic mortality for example, will regulate population density, a well-established demographic feature that modulates disease spread. However, extrinsic mortality will shape other population characteristics such as age structure. The age structure of a population could affect disease dynamics through a number of mechanisms; here, we consider (1) when individuals of different ages show different susceptibilities (i.e. age effects), or (2) when the offspring of

mothers of different ages show different susceptibilities (i.e. maternal age effects).

The age of a host at exposure to a parasite is well established to affect the probability and severity of infection, this is partly due to the ontogeny of the immune system (Lesser et al., 2006, Nussey et al., 2008, Hasselquist & Nilsson, 2009). In vertebrates this includes the development of adaptive immunity followed by immunosenescence, but invertebrates also appear to show a clear development of the immune system, or pathogen resistance (Rheins & Karp, 1985, Wilson-Rich et al., 2008, Piñera et al., 2013, Garbutt et al., 2014a, Izhar & Ben-Ami, 2015, Izhar et al., 2015). The consideration of age related effects on epidemiological processes has contributed greatly to disease prevention strategies (Anderson & May, 1982a, Katzmann & Dietz, 1984, Müller, 2000).

By contrast, maternal age effects on offspring susceptibility to pathogens have received little attention. Generally, maternal effects have been shown to affect population dynamics and demography through alterations in offspring reproduction, maturation and growth rate (Gaillard et al., 2003, Benton et al., 2005, Beamonte-Barrientos et al., 2010) and maternal effects have been shown to alter population robustness in the face of ecological challenges (Räsänen & Kruuk, 2007, Kuijper & Hoyle, 2015). The maternal condition, for example nutrition availability or disease status, is increasingly recognised to affect offspring susceptibility to infection (Huang & Song, 1999, Little et al., 2003, Mitchell & Read, 2005, Gasparini et al., 2007, Lorenz & Koella, 2011, Stjernman & Little, 2011, Tidbury et al., 2011, Boots & Roberts, 2012, Garbutt & Little, 2017). This suggests variation in phenotypic traits, as a response to the maternal environment, can drive

population and epidemiological dynamics (Beckerman et al., 2002, Mitchell & Read, 2005, Garbutt et al., 2014b).

Maternal age has been shown to affect offspring performance as offspring of older mothers are often born larger and mature at a greater size (Priest et al., 2002, Benton et al., 2008). However, although older mothers produce larger offspring, they tend to produce fewer of these, suggesting they are subject to a 'size versus number' trade-off. Large *Daphnia* offspring have been shown to be more resistant to infection than smaller offspring (Garbutt & Little, 2017), suggesting the effect of maternal age on offspring body size may influence pathogen susceptibility. Furthermore, maternal effects on offspring may alter the competitive environment experienced by successive generations through for example, body size effects or increase population sizes (Beckerman et al., 2006, Kindsvater et al., 2011, Prior et al., 2011). If the competitive environment regards nutrient availability, maternal effects may indirectly alter disease dynamics as nutrient availability can alter both host susceptibility to infection and pathogen distribution (Vale et al., 2013, Stahlschmidt et al., 2015, Cotter et al., 2011).

To understand how maternal age influences susceptibility, we studied five clutches of offspring from the parthenogenic crustacean *Daphnia magna* when exposed to a bacterial pathogen. In addition to pathogen resistance, we collected data on reproduction. The experiment showed that older mothers give birth to more resistant offspring. Earlier work from our laboratory (Garbutt et al., 2014a) and others (Izhar & Ben-Ami, 2015, Izhar et al., 2015) showed that older mothers themselves are more resistant to this pathogen. We used these two observations – that older mothers are both more resistant and give birth to offspring that are more resistant - to develop

a compartmental model describing how population age structure, the effects of age and maternal effects combine to influence the spread of infection.

Study System

Daphnia magna (Crustacea: Cladocera) are filter feeding planktonic crustaceans found in small freshwater ponds. *Pasteuria ramosa* is a sterilizing specialist bacterial pathogen of *Daphnia magna* that is transmitted horizontally when spores released from infected cadavers are ingested by uninfected filter feeding hosts (Ebert et al., 2016, Ebert et al., 1996). In this experiment we used a *Daphnia* clone (Kc49a) from a population in a pond in Kaimes in the Scottish borders, and a *P. ramosa* strain isolated from the same population. This host clone was part of a study of genetic variation in maternal effects (Stjernman & Little, 2011) and shows a response that is typical of its population (see Little & Colegrave, 2016 for discussion of the merits of single versus multi-genotype studies).

Methods

The basic design of our experiment was to measure the pathogen susceptibility of offspring from first, second, third and fifth clutches. Two new-born *Daphnia* were isolated from each clutch. One of these offspring was exposed to *P. ramosa*. The second offspring was used for measurement of reproduction. Each new-born also had their body size measured before being placed into their treatment groups.

Acclimation

Twenty-four replicates, each an individual *Daphnia* in a 60 ml glass jar, were acclimatised for three generations under standardised conditions. This process is designed to equilibrate uncontrolled maternal effects and ensure that each replicate is independent. During this time *Daphnia* were kept in artificial pond medium (Kluttgen et al., 1994) in an incubator with a light:dark cycle of 12:12 L:D at 20°C. They were fed daily with 7×10^6 cells of green algae, *Chlorella* spp, and changed into fresh medium twice a week and when offspring were present in the jar. Each new generation was initiated with offspring from the second clutch. After the third homogenising generation one individual was taken from each replicate and placed in fresh jars to be the mothers of the experimental animals. These individuals were kept in the same conditions as the homogenising generations.

Experimental generation

Experiment 1

Offspring from clutch 1, 2, 3, and 5 of the maternal generation were our experimental animals. Two offspring were taken from each mother and were randomly assigned to either pathogen exposure or control - used for the measurement of fecundity in the absence of infection. For pathogen exposure, 20,000 *P. ramosa* spores were added to each jar and media was not changed for five days. Spore mixes were prepared earlier by crushing infected *Daphnia* and counting spores with a haemocytometer. At the end of the five-day exposure treatment, *Daphnia* were changed into clean jars with fresh media. *Daphnia* were maintained for 28 days after the exposure period under the conditions used for acclimation. During this period the date of birth of each clutch and number of individuals born to each clutch was recorded. At the end of the 28-day period, infections were diagnosed

(infections are easy to discern with the naked eye as *Daphnia* have a clear carapace and reddish-brown bacterial growth is visible in the haemolymph in addition to the lack of reproduction). The control *Daphnia* whose fecundity was measured were handled identically, except they were not exposed to parasite spores. All newborn *Daphnia* were photographed for later measurement of size at birth.

Independent Replication of experiment 1

The design and methodology of this experiment was identical to that detailed above: we measured the fitness of offspring from first, second, third and fifth clutches following identical protocol for pathogen exposure, and control individuals.

Analysis

Body size at birth and total reproduction were analysed in a General Linear Model. Age at first reproduction was studied with a parametric survival analysis following a Weibull distribution. A generalised linear model (with binomial errors and logit link function) was used to study the probability of becoming infected. In all cases the explanatory variable was the clutch the *Daphnia* originated from as this acts as our proxy for maternal age. The different response variables were analysed with different subsets of the data: size at birth considered the entire data set, reproduction considered only *Daphnia* that were unexposed to *P. ramosa*, while the probability of infection included only *Daphnia* that were exposed to the pathogen.

Experimental Results

The age of the mother had a significant effect on the probability of offspring infection ($\chi^2 = 24.8$, $p < 0.0001$) where offspring from young mothers were

highly susceptible to infection, as shown in Figure 2.1A. Maternal age (or variable “Clutch” for clutch number) also had a significant effect on total reproduction ($f_{3,72} = 15.8$, $p < 0.0001$) as seen in 2.1B and size at birth as seen in Figure 2.1C ($f_{3,91} = 219$, $p < 0.0001$). Age at first reproduction was not influenced by maternal age ($\chi^2 = 5.07$, $p = 0.16$). These patterns were confirmed through the independent replication of the experiment. Maternal age had a significant effect on the probability of becoming infected ($\chi^2 = 20.6$, $p < 0.0001$; Figure 2.1A), total reproduction ($f_{3,76} = 3.91$, $p < 0.002$; Figure 2.1B), and size at birth ($f_{3,105} = 96$, $p < 0.0001$; Figure 2.1C).

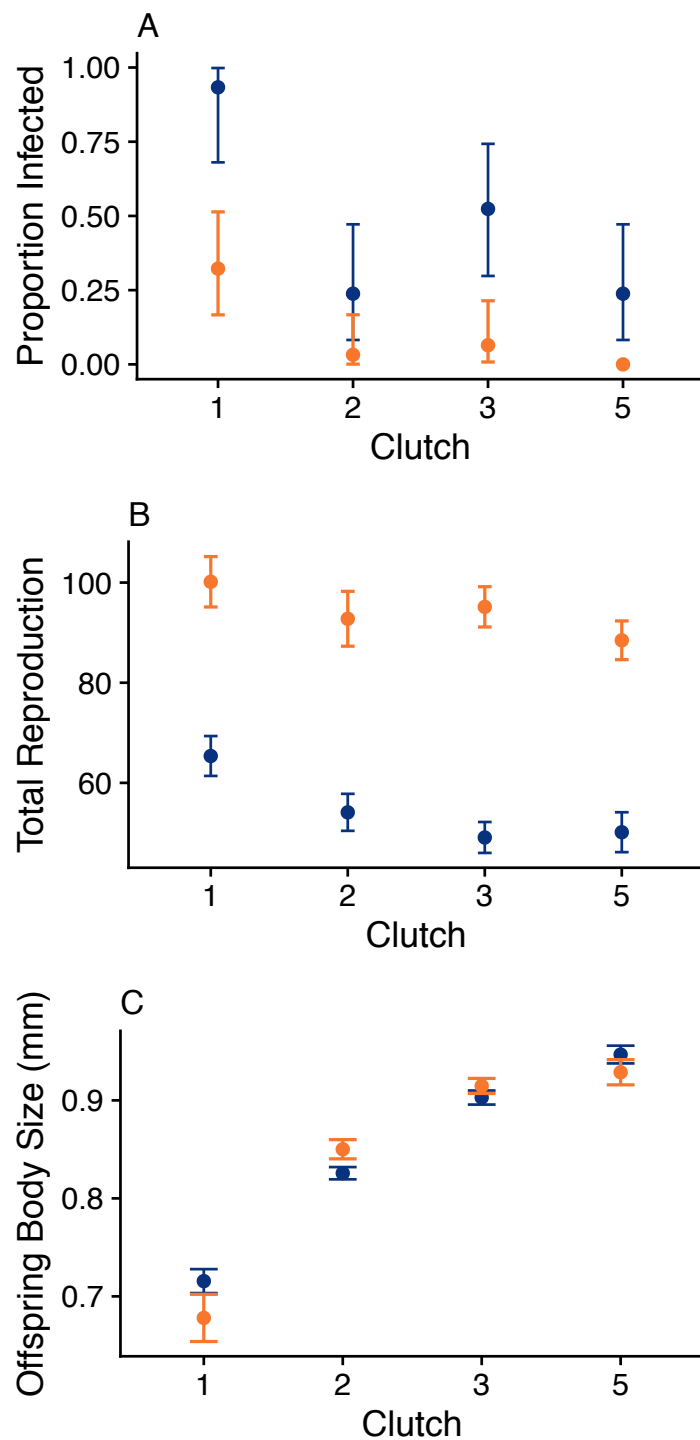


Figure 2.1. Both the original experiment (shown in blue) and independent replication of the experiment (shown in orange) showing (A), proportion of infections resulting from exposure of the treatments groups from clutches 1, 2, 3 & 5, (B) reproductive output and (C) the effect of clutch number (maternal age at reproduction) on offspring body size.

Epidemiological model

Our experimental results showed a strong effect of maternal age on offspring resistance, with older mothers producing more resistant offspring. Previous research showed that *D. magna* show age-specific susceptibility, where older individuals are more resistant to infection than young individuals (Garbutt et al., 2014a, Izhar & Ben-Ami, 2015, Izhar et al., 2015). Host population demography and environmental factors such as baseline mortality rates, predation and density have been shown to influence transmission rates and pathogen virulence evolution. Pathogen transmission is a function of an infected individuals' rate of contact with other potential hosts. As increased host population density should increase contact rates, it is generally expected that an increase in population density will result in an increase in transmission potential (Anderson & May, 1979, Ebert & Mangin, 1997, Choo et al., 2003). The theoretical consideration of age-specific effects on epidemiological dynamics, tends to consider age-specific mortality and age-specific contact rates rather than the strength or weakness of an age-associated immune response (Anderson & May, 1982c, Katzmann & Dietz, 1984, Castillo-Chavez et al., 1989, Müller, 2000). In addition to this, the presence of maternal age effects on offspring susceptibility has not been considered. We therefore developed a compartmental model incorporating these age effects on the expected number of secondary infections resulting from the introduction of an individual infected with a novel pathogen into a completely susceptible population (R_0). R_0 depends on the duration of infection, the probability of infecting a susceptible during one contact, and the rate of contact per unit of time (Dietz, 1993). The output value can be used as a benchmark. If the value of this is >1 then the disease will spread (Anderson & May, 1979).

We divide the population into four age classes, each of which could be infected (I) or uninfected (U), giving a total of eight classes of individuals in the model – $U_{Y,Y}$, $U_{Y,O}$, $U_{O,Y}$, $U_{O,O}$, $I_{Y,Y}$, $I_{Y,O}$, $I_{O,Y}$, $I_{O,O}$ (figure 2). Here, the first subscript indicates the individual's age (Y for young, O for old), and the second subscript indicates the individual's mother's age at that individual's birth. The parameters in the model are: β is the baseline transmission rate between infected and uninfected individuals; δ is the baseline death rate; α is pathogen virulence as measured by disease induced death; ρ is the probability that non-pathogen induced death of an infected individual will also lead to transmission; m is the rate of maturation (i.e. The rate at which individuals move from the young age classes to the old); M describes the proportional reduction in an individual's susceptibility as associated by having a mother of the age class "Old" applied to the baseline β ; A describes the proportional reduction to an individual's susceptibility by themselves being of the age class "Old" applied to the baseline β ; r is the maximum per capita growth rate of hosts; K is the carrying capacity of the host population, controlling density dependent limitation on reproduction. The transmission terms, as shown in figure 2.2, contain the rates of death, due to *P. ramosa* being transmitted only upon host death. For the sake of simplicity, we make the assumption that there is a constant rate of death across age groups. We do however assume that susceptibility varies between these classes, and that reproduction is resource limited with a constant maximum reproductive rate per day for uninfected individuals. We assume that infected individuals do not reproduce as *P. ramosa* castrates the host during infection (Ebert et al., 1996).

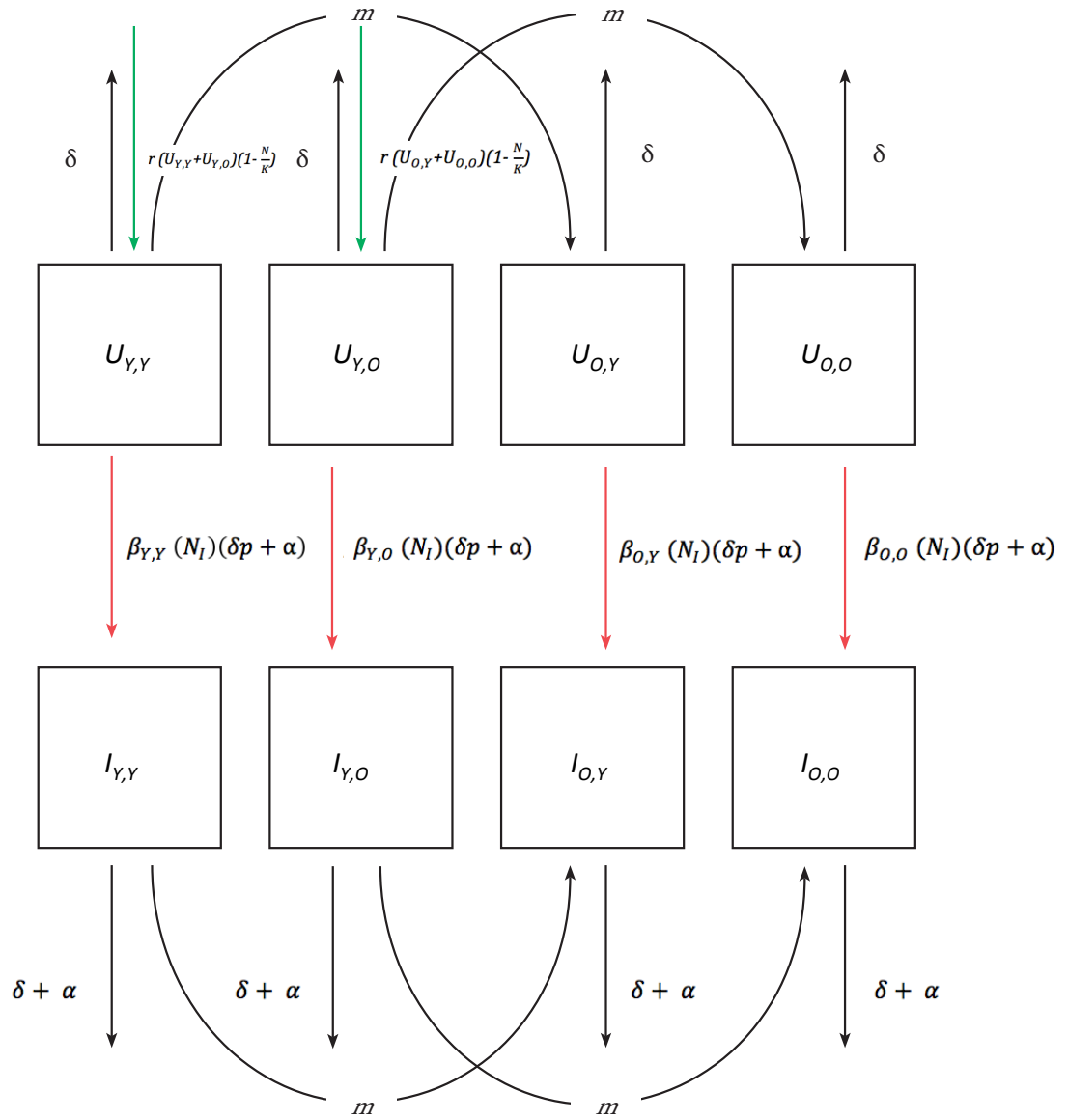


Figure 2.2. The compartmental model with uninfected and infected groups of four age-classes. $U_{Y,Y}$ – Young individuals with a young mother. $U_{Y,O}$ – young individuals with an old mother. $U_{O,Y}$ – Old individuals with a young mother. $U_{O,O}$ – Old individuals with an old mother. m is the rate of maturation; β is the transmission rate. δ is baseline mortality, α is pathogen induced mortality. Other parameters are r - maximum reproduction; K - carrying capacity; N - total population number. As *P. ramosa* is only transmitted upon host death, death rates are included in the transmission terms.

In the absence of the pathogen we write the dynamics of the four uninfected age classes as

$$\frac{dU_{Y,Y}}{dt} = r(U_{Y,Y} + U_{Y,O}) \left(1 - \frac{N_U}{K}\right) - (m + \delta)U_{Y,Y} \quad 1a$$

$$\frac{dU_{Y,O}}{dt} = r(U_{O,Y} + U_{O,O}) \left(1 - \frac{N_U}{K}\right) - (m + \delta)U_{Y,O} \quad 1b$$

$$\frac{dU_{O,Y}}{dt} = mU_{Y,Y} - \delta U_{O,Y} \quad 1c$$

$$\frac{dU_{O,O}}{dt} = mU_{Y,O} - \delta U_{O,O} \quad 1d$$

$$N_U = U_{Y,Y} + U_{Y,O} + U_{O,Y} + U_{O,O} \quad 1e$$

The equilibrium densities of each age class are given by

$$U_{Y,Y} = \frac{(r\delta^2 - \delta^3)K}{r(\delta + m)^2} \quad 2a$$

$$U_{Y,O} = \frac{\delta m(r - \delta)K}{r(\delta + m)^2} \quad 2b$$

$$U_{O,Y} = \frac{\delta m(r - \delta)K}{r(\delta + m)^2} \quad 2c$$

$$U_{O,O} = \frac{m^2(r - \delta)K}{r(\delta + m)^2} \quad 2d$$

Here we see that the equilibrium density of $U_{O,O}$ decreases with increasing mortality δ (as $r > \delta$ is necessary for a positive equilibrium). The equilibrium density of both $U_{Y,O}$ and $U_{O,Y}$ initially increases with increasing mortality before decreasing once $\delta > 2r + m$. Similarly, the density of the age class $U_{Y,Y}$ initially increases with increasing mortality before decreasing once $\delta > (\sqrt{m(9m + 8r)} - 3m)/2$ (Figure 2.3).

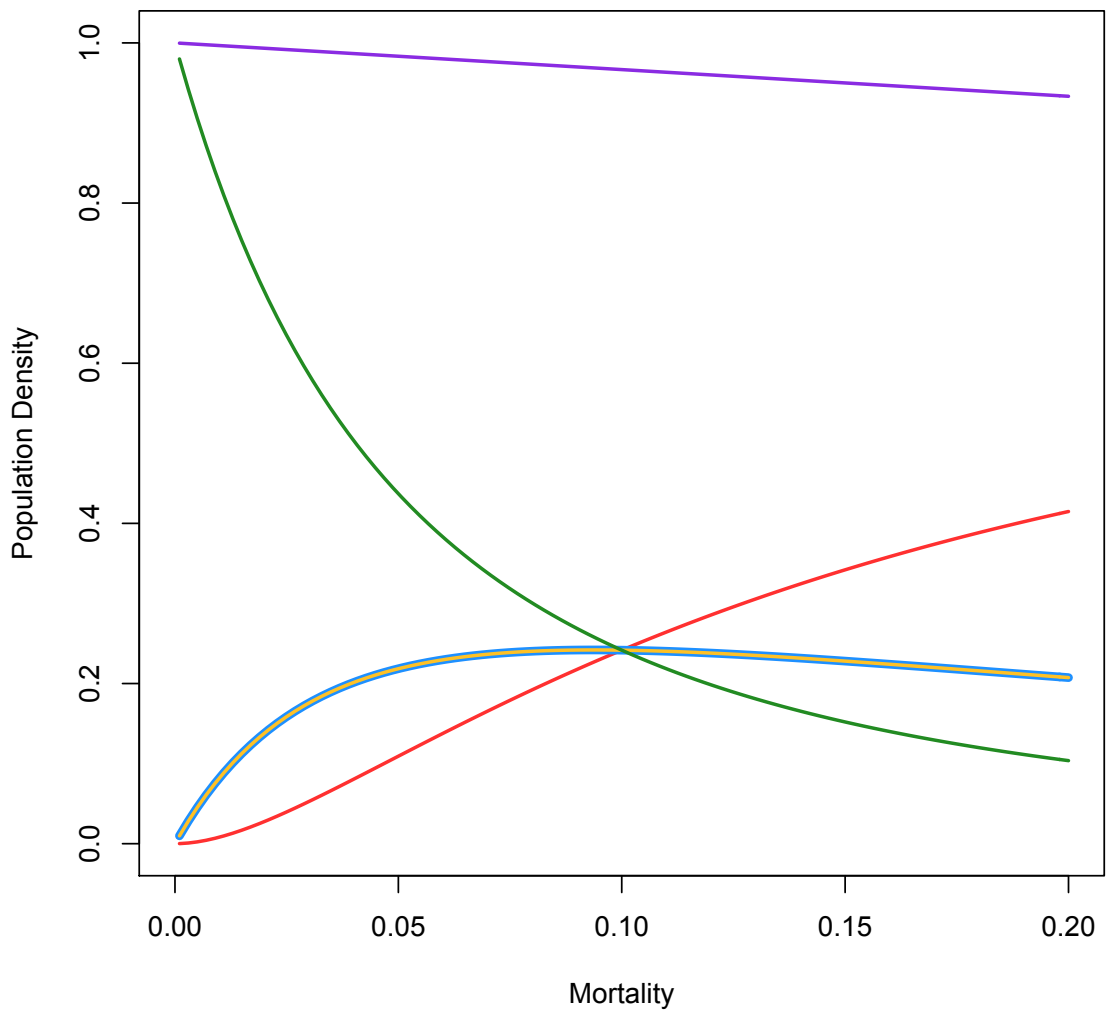


Figure 2.3. Showing the changes in total population density with increased mortality (purple line). This is broken down into the age classes showing density of young individuals with young mothers (red line), young individuals with old mothers (yellow line), old individuals with young mothers (blue line) and old individuals with old mothers (green line). Due to parameterization of the model the yellow and blue lines trace one another. Other parameters are $r = 3$; $K = 1$; $m = 1/10$.

This bias of the age structure towards more susceptible younger individuals and individuals from younger mothers occurs as increased mortality frees up resources for reproduction, resulting in the production of more young individuals who will themselves reproduce leading to more individuals from young mothers. At this equilibrium the total density of uninfected hosts, N_U , is

$$N_U = K \left(1 - \frac{\delta}{r}\right) \quad 3$$

meaning that the total density of uninfected individuals monotonically declines with increasing mortality rate (Figure 2.3).

The population dynamics in the presence of the pathogen are given by

$$\frac{dU_{Y,Y}}{dt} = r(U_{Y,Y} + U_{Y,o}) \left(1 - \frac{N}{K}\right) - (m + \delta)U_{Y,Y} - \beta_{Y,Y}(\alpha + p\delta)N_I U_{Y,Y} \quad 4a$$

$$\begin{aligned} \frac{dU_{Y,o}}{dt} = & r(U_{o,Y} + U_{o,o}) \left(1 - \frac{N}{K}\right) - (m + \delta)U_{Y,o} \\ & - \beta_{Y,o}(\alpha + p\delta)N_I U_{Y,o} \end{aligned} \quad 4b$$

$$\frac{dU_{o,Y}}{dt} = mU_{Y,Y} - \delta U_{o,Y} - \beta_{o,Y}(\alpha + p\delta)N_I U_{o,Y} \quad 4c$$

$$\frac{dU_{o,o}}{dt} = mU_{Y,o} - \delta U_{o,o} - \beta_{o,o}(\alpha + p\delta)N_I U_{o,o} \quad 4d$$

$$\frac{dI_{Y,Y}}{dt} = \beta_{Y,Y}(\alpha + p\delta)N_I U_{Y,Y} - (m + \alpha + \delta)I_{Y,Y} \quad 4e$$

$$\frac{dI_{Y,o}}{dt} = \beta_{Y,o}(\alpha + p\delta)N_I U_{Y,o} - (m + \alpha + \delta)I_{Y,o} \quad 4f$$

$$\frac{dI_{o,Y}}{dt} = \beta_{o,Y}(\alpha + p\delta)N_I U_{o,Y} - (\alpha + \delta)I_{o,Y} + mI_{Y,Y} \quad 4g$$

$$\frac{dI_{o,o}}{dt} = \beta_{o,o}(\alpha + p\delta)N_I U_{o,o} - (\alpha + \delta)I_{o,o} + mI_{Y,o} \quad 4h$$

$$N_U = U_{Y,Y} + U_{Y,O} + U_{O,Y} + U_{O,O} \quad 4i$$

$$N_I = I_{Y,Y} + I_{Y,O} + I_{O,Y} + I_{O,O} \quad 4j$$

$$N = N_U + N_I \quad 4k$$

Note that as *P. ramosa* transmits at host death, transmission between infected and uninfected hosts is weighted by $(\alpha + p\delta)$, where α is the pathogen induced death rate, which is assumed to always facilitate transmission, and p is the proportion of non-pathogen induced death that also facilitates transmission.

To calculate the R_0 of the pathogen we consider the number of secondary infections caused by a rare pathogen at the pathogen-free equilibrium given in equations 2a-2d.

$$R_0 = \frac{(\alpha + p\delta)(\beta_{Y,Y}U_{Y,Y} + \beta_{Y,O}U_{Y,O} + \beta_{O,Y}U_{O,Y} + \beta_{O,O}U_{O,O})}{\alpha + \delta} \quad 5$$

To evaluate the effects of age-class specific susceptibilities on the R_0 we define the effects of age-related and maternal age-related reduction in susceptibility as

$$\beta_{Y,Y} = \beta \quad 6a$$

$$\beta_{O,Y} = \beta(1 - A) \quad 6b$$

$$\beta_{Y,O} = \beta(1 - M) \quad 6c$$

$$\beta_{O,O} = \beta(1 - A)(1 - M) \quad 6d$$

where $0 \leq A \leq 1$ and $0 \leq M \leq 1$ are the proportional reductions in susceptibility owing to age and maternal age respectively, which here we assume to interact multiplicatively (though see later for additive effects).

This yields a pathogen R_0 of

$$R_0 = \frac{\beta(r - \delta)(\alpha + \delta p)(\delta + m(1 - M))(\delta + m(1 - A))K}{r(\alpha + \delta)(\delta + m)^2} \quad 7$$

To evaluate the effect of non-pathogen induced mortality, δ , on the pathogen R_0 we first consider the derivative of R_0 with respect to δ as δ approaches infinity, which gives

$$\lim_{\delta \rightarrow \infty} \frac{dR_0}{d\delta} = -\frac{\beta p K}{r} \quad 8$$

which is strictly negative, meaning that at very high values of δ the pathogen R_0 must decline as the host population approaches zero. Similarly, if we consider the behaviour of this derivative as δ approaches zero we get

$$\begin{aligned} \lim_{\delta \rightarrow 0} \frac{dR_0}{d\delta} = \frac{\beta K}{\alpha m r} & \left(\alpha(r(A + M - 2AM) - m(1 - A)(1 - M)) \right. \\ & \left. - mr(1 - A)(1 - M)(1 - p) \right) \end{aligned} \quad 9$$

which is positive whenever

$$m < \frac{\alpha r(A + M - 2AM)}{(1 - A)(1 - M)(\alpha + r(1 - p))} \quad 10$$

This inequality can never be satisfied for $A = 0$ and $M = 0$, meaning that in the absence of age and maternal age-based reductions in susceptibility the pathogen R_0 always decreases with increasing mortality. However, this inequality can be satisfied for sufficiently large reductions in susceptibility with age and maternal age (high A and M), meaning that pathogen R_0 can initially increase with non-pathogen induced mortality. This humped relationship can be explained as follows. In the absence of reductions in susceptibility with age and maternal age, reductions in host population density with increasing mortality reduce pathogen R_0 owing to reduced

transmission. However, increased mortality also shifts the age structure of the population towards younger individuals and individuals from younger mothers, which can increase in density even as the total population density declines. If these age classes of individual are sufficiently more susceptible to infection, this increase in their density can more than compensate for the total reduction in host population density, leading to an increase in R_0 with increasing non-pathogen induced mortality. However, at very high mortality rates the total host population density is sufficiently reduced that shifts in age structure no longer compensate, and R_0 declines with extrinsic mortality, resulting in a humped relationship. Transmission potential begins to decline at lower mortality rates when, p (the probability that non-pathogen induced death of an infected individual will lead to transmission) is low, as increasing mortality will result in loss of infections with little opportunity for transmission. Note however that our qualitative results hold even in the case of $p = 0$. The relationship is illustrated with and without both the age and maternal age-related reductions in susceptibility in Figure 2.4.

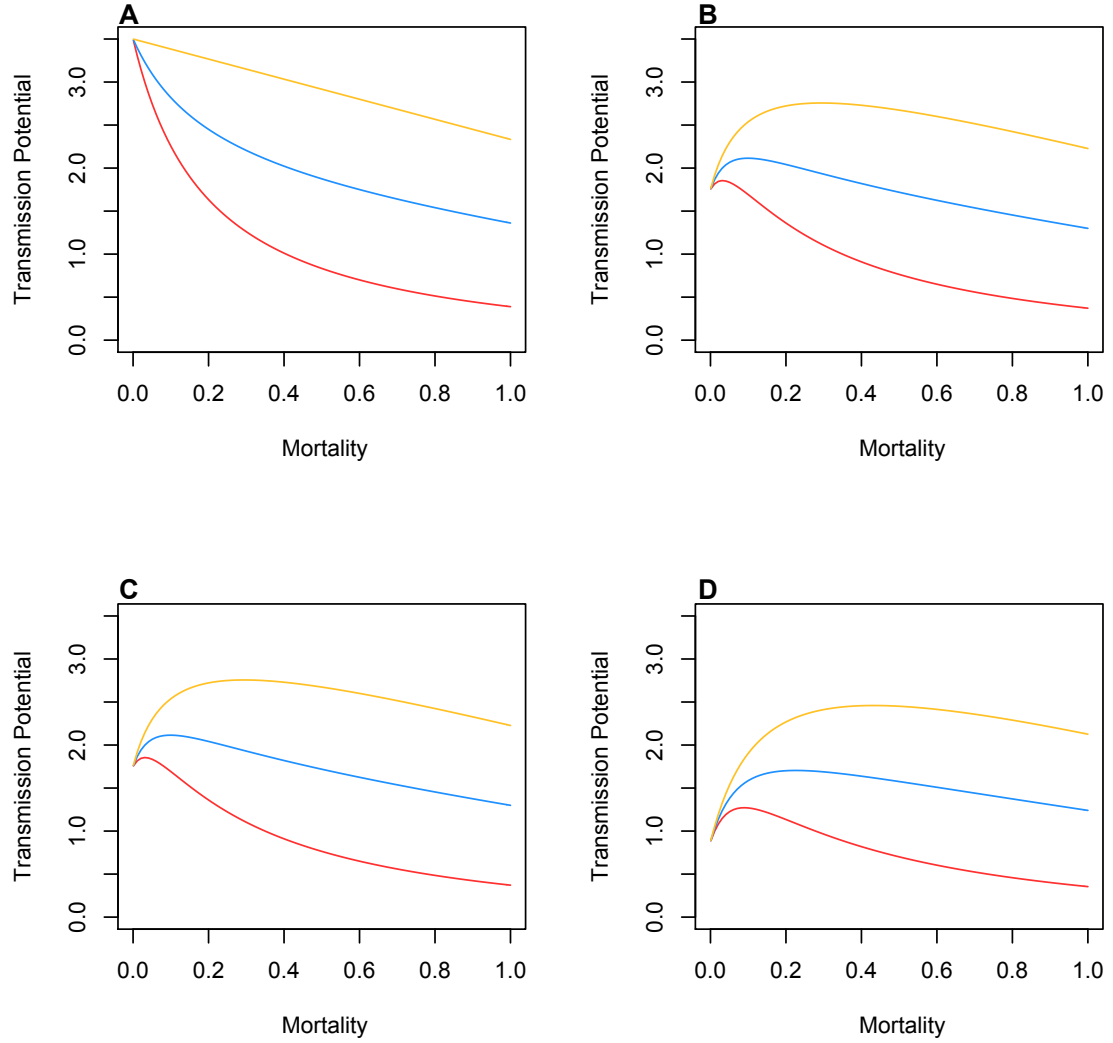


Figure 2.4. Modelling outputs of the relationship between mortality and transmission potential (R_0) with $p=1$ (yellow line), $p=0.5$ (blue line), and $p=0$ (red line). A: No age effects or maternal age effects present, B: Maternal age effects, C: Age specific susceptibility, or D: Both maternal and age effects present. In the presence of no effects (A), the expected negative relationship between mortality and transmission potential is shown at all levels of extrinsic mortality. The presence of maternal age effects (B), age specific susceptibility (C) or both effects (D) within a population results in a humped relationship, where an increase in mortality initially increases transmission, due to the shift in density of susceptible individuals. This positive relationship between mortality and transmission potential is most pronounced at all levels of extrinsic mortality when both effects are present. Other parameters are: $r = 3$; $K = 1$; $a = 1/5$; $m = 1/10$; $\beta_{Y,Y} = 3.5$

In equation 6 we assume that age and maternal age interact multiplicatively. Here we show that our qualitative results also hold for additive effects. In this case the susceptibilities for each age class are

$$\beta_{Y,Y} = \beta \quad 11a$$

$$\beta_{O,Y} = \beta(1 - A) \quad 11b$$

$$\beta_{Y,O} = \beta(1 - M) \quad 11c$$

$$\beta_{O,O} = \beta(1 - A - M) \quad 11d$$

Now the number of expected secondary infections caused by a rare infection is

$$R_0 = \frac{\beta(r - \delta)(\alpha + \delta p)(\delta + m(1 - M - A))K}{r(\alpha + \delta)(\delta + m)^2} \quad 12$$

We can again calculate the derivative of R_0 with respect to δ as δ approaches infinity, which gives

$$\lim_{\delta \rightarrow \infty} \frac{dR_0}{d\delta} = -\frac{\beta p K}{r} \quad 13$$

which is again strictly negative, meaning that at very high values of δ the pathogen R_0 must decline as the host population approaches zero.

Similarly, if we consider the behaviour of this derivative as δ approaches zero we get

$$\lim_{\delta \rightarrow 0} \frac{dR_0}{d\delta} = \frac{\gamma \beta K}{\alpha m r} \left(\alpha(r(A + M) - m(1 - A - M)) - mr(1 - A - M)(1 - p) \right) \quad 14$$

which is positive whenever

$$m < \frac{\alpha r(A + M)}{(1 - A - M)(\alpha + r(1 - p))} \quad 15$$

Again, this inequality can never be satisfied for $A = 0$ and $M = 0$, meaning that in the absence of age and maternal age-based reductions in susceptibility the pathogen R_0 always decreases with increasing mortality.

However, this inequality can be satisfied for sufficiently high values of A and M , meaning that pathogen R_0 can initially increase with non-pathogen induced mortality, matching our qualitative results with multiplicative effects.

Discussion

Our experiment makes the novel observation that younger *D. magna* mothers give birth to offspring that are more pathogen susceptible, when exposed to *P. ramosa*. This data combined with recent observations that younger hosts are themselves more susceptible (Garbutt et al., 2014a, Izhar et al., 2015, Izhar & Ben-Ami, 2015) formed the basis of a compartmental model showing that increasing extrinsic mortality, and thus decreasing total host population density, can lead to increasing transmission. The relationship between mortality and transmission that is classically seen, where higher host densities generate greater transmission, is so pervasive it has become a central assumption of the epidemiological theory that contributes to our understanding of pathogen traits and virulence (Anderson & May, 1979, Read, 1994, Alizon et al., 2009). Thus, we have identified a new, and biologically realistic context in which age effects and maternal effects combine to alter the ecological conditions favouring disease transmission.

Our observed effect of maternal age on offspring performance is an example of maternal effect-driven, delayed life history effects (Beckerman et al., 2002) that lead to whole cohort effects (Ginzburg et al., 1994, Lindström, 1999). The maternal condition has previously been shown to have prolonged effects on offspring fecundity, maturation and growth rate over successive generations, influencing population dynamics (Gaillard et al., 2003, Benton et al., 2005, Beamonte-Barrientos et al., 2010). We now add

offspring susceptibility to this list and can infer from our model that maternal age effects on susceptibility can then impact successive cohorts, by altering the condition of each cohort at reproduction. Considering *P. ramosa* specifically, the predictions of our model are biologically feasible. This pathogen sterilizes hosts, but often one clutch is produced before reproduction stops. If no effect of maturation or maternal effects were present, the transmission potential would decrease as seen in figure 4. In the presence of these effects, however, those who are young when infected only reproduce once whilst young and then do not contribute to the population at a later age. Their offspring are therefore expected to be the most susceptible. As mortality increases, the density of young individuals contributing highly susceptible individuals to the population increases, resulting in the dynamic we show.

The maternal effect of increased resistance in offspring born to older mothers could be explained by size at birth. Older mothers are known to produce larger offspring (Marshall et al., 2010, Kindsvater et al., 2011) and therefore the size at birth could be responsible to some degree for the resistance to disease. This correlation of body size to susceptibility has been made before in *Daphnia*, and it has been suggested this is due to increased resource availability for costly immune defences and somatic maintenance (Garbutt et al., 2014a). In *Daphnia* spp. and other systems however, this correlation between increased size and reduced infection probability does not always hold true (Hall et al., 2007b, Stjernman & Little, 2011). Furthermore, the assumption that increased body size equates to better fitness and performance doesn't explain how smaller offspring of young mothers are capable of reproducing in higher numbers than their larger counterparts.

The observed trade-off between offspring size and the decreasing number of offspring in each successive reproductive event could be adaptive if clutches of older mothers were to experience more challenging environments than young mothers. This is plausible given population size could increase, increasing competition, and potentially pathogen prevalence. It has been seen previously that *Daphnia* produce larger and more resilient offspring in tough conditions (Mitchell & Read 2005; Garbutt et al. 2014b) and larger individuals are better competitors for resources in a more dense populations (Brockelman 1975). In addition, individuals born to older mothers may need to be larger in order to compete with their older siblings (Plaistow et al. 2007).

In summary, our experimental results add evidence to a growing body of work showing maternal age effects on offspring performance (Berkeley et al. 2004; Plaistow et al. 2007; Marshall et al. 2010). Furthermore, our model shows that where age-related and maternal age-related effects occur, they can fundamentally transform the ecological conditions that favour disease transmission. Divergent host susceptibilities within a population could also have marked effects on pathogen evolution and virulence, and our model lends itself to an extension that includes studies of optimal virulence.

Chapter 3 Pathogen Dynamics Across the Diversity of Ageing

Formatted for submission to PNAS for publication, Reading order: Summary, Introduction, Experimental Results, Model Results, Methods.

Summary

Reproduction, mortality and immune function often change with age, but they do not invariably deteriorate. Across the tree of life, there is extensive variation in age-specific performance and changes to key life-history traits (Jones et al., 2014). These changes occur on a spectrum from classic senescence, where performance declines with age, to juvenescence, where performance improves with age. Reproduction, mortality and immune function are also important factors influencing the spread of infectious disease, yet there exists no comprehensive investigation into how the ageing spectrum of these traits impacts epidemics. We used a model laboratory infection system to compile an ageing profile of a single organism, including traits directly linked to pathogen resistance, and those that should indirectly alter pathogen transmission by influencing demography. In doing so we demonstrate the extent of asynchrony in ageing across traits within a single organism. We then developed generalisable epidemiological models demonstrating that different patterns of ageing produce dramatically different transmission landscapes: in many cases ageing can reduce the probability of epidemics, though it can also promote severity. This work provides context and tools for use across taxa by empiricists, demographers and epidemiologists, advancing our ability to accurately predict factors contributing to epidemics, or the potential repercussions of senescence manipulation.

Introduction

Global populations are ageing at an unprecedented rate (He et al., 2016). This is a concern as pathogen dynamics are a function of host reproduction, mortality and susceptibility to infection, which in turn are functions of host age. In the face of this rapid change, it is therefore surprising that population age structure is still largely considered in an epidemiological context often as simply age-specific contact rates (Mossong et al., 2008, Wallinga et al., 2006). To develop a greater appreciation for the effects of individual ageing at the population level however, a more in depth understanding of age-related change in physiological performance is required. This is challenging as comprehensive investigations into whole organism ageing, beyond reproduction and mortality are limited and consider reproduction only as fecundity, despite a large body of evidence suggesting maternal condition influences offspring performance (Boots & Roberts, 2012, Garbutt & Little, 2017, Kaneko et al., 2011, Mitchell & Read, 2005). Furthermore, evidence would suggest that longstanding predictions of ageing theory are not supported by data. For example, the dogma of ageing theory states (Gaillard & Lemaître, 2017, Williams, 1957) that the process of ageing should result in a decline in physiological capability, and that the force of selection will act to synchronise this deterioration across traits within an organism. But there is substantial evidence of ageing occurring on a spectrum, seeing some organisms juvenescence as their physiological performance improves with age, whilst others may senesce, as performance declines with age (Jones et al., 2014). There is also clear evidence of asynchrony in the age-specific onset and rate of age-related change in a range of performance measures (Lecomte et al., 2010, Massot et al., 2011, Hayward et al., 2015, Bansal et al., 2015). The extent of this

asynchrony, however, remains thoroughly underexplored and is therefore poorly characterised.

Given the parallels between ageing and epidemiology and the ubiquity of population age structure in natural populations, understanding how ageing affects epidemiological dynamics is a pressing question, applicable across taxa. We therefore addressed this with two approaches. First, utilising the tractable model laboratory infection system, *Daphnia magna* and its bacterial pathogen *Pasteuria ramosa*, we compiled an ageing profile, collecting data on key life-history traits. The use of the longstanding, non-pharmacological ageing intervention Dietary Restriction (DR), decreases the rate of senescence, increasing the lifespan of many organisms at the expense of reducing reproductive output (Good & Tatar, 2001, Colman et al., 2009, Adler & Bonduriansky, 2014, Austad, 1989, Austad, 1993). We therefore applied DR to manipulate the rate at which maternal *Daphnia* aged, to delineate the effects of maternal age and maternal senescence on offspring performance. We then developed general epidemiological models applicable across taxa in order to test the following two hypotheses based on our experimental observations: 1. That age-specific pathogen susceptibility and trans-generational pathogen susceptibility, or other physiological characteristics of age that alter a host's susceptibility to infectious disease, should directly impact infection dynamics through alterations to the occurrence of transmission events. 2. That changes in rates of mortality or reproduction as a result of age will indirectly alter infectious disease burden by altering population density, demography and the supply of susceptible hosts. These hypotheses were tested using a deterministic age structured model (Clark et al., 2017) that explicitly considered the full spectrum of ageing from juvenescence to senescence.

Ageing traits were modelling in line with a pleiotropy model of ageing (Williams, 1957, Garbutt et al., 2014a), where increased performance in a trait at one age results in decreased performance for that same trait in the opposing age class. We investigated the effect of age-related physiological changes on pathogen transmission potential, R_0 , a benchmark value that determines epidemic potential within a given population. If a pathogen R_0 is > 1 then its introduction to a given population will result in a sustained outbreak (Dietz, 1993). Through these two approaches we show that the characterisation of ageing requires more than mortality and fecundity measures, and that ageing is indeed a multifaceted architect of pathogen dynamics, requiring far greater consideration in epidemiological studies.

Asynchronous Ageing

Consistent with classic ageing theory (Medawar, 1952), the probability of mortality increased with age in *D. magna* when modelled with a Weibull distribution (methods), as chosen by AIC comparison (Fig. 3.1A; shape (α) = 5.03, 95% CI lower = 4.52, upper = 5.60; scale (μ) = 79.55% CI lower = 79.10, upper = 83.16). Infection with the pathogen *Pasteuria ramosa* reduced survival by 35% (Fig. 3.1A; estimate = 0.65; 95% CI lower = 0.61, upper = 0.69), despite increased resistance to infection with age (Garbutt et al., 2014a, Izhar & Ben-Ami, 2015). In line with both theory (Adler & Bonduriansky, 2014, Holliday, 1989) and numerous empirical observations of diet and ageing (McCay et al., 1935, Klass, 1977, Austad, 1989), calorie restriction increased *D. magna* longevity, in comparison to normally fed females (Fig. 3.1A; estimate = 1.0989, 95% CI lower = 1.0404, upper = 1.1607). These longer living females also had lower rates of reproductive senescence with an overall lower number of offspring produced in each

clutch, but with the number of offspring remaining consistent over time (Fig. 3.1B; linear interaction $f_{1, 1337.1} = 70.73$, $p = <0.0001$, quadratic interaction $f_{1, 1298.7} = 10.07$, $p = 0.0015$). Despite senescence of offspring quantity from normally fed mothers, offspring quality showed juvenescence. Being a general indicator of quality (Gaillard & Lemaître, 2017, Garbutt & Little, 2017, Metcalfe & Monaghan, 2001, Ebert, 1994), we measured offspring body size and found that older mothers bear larger offspring, with caloric restriction only affecting the size of offspring from primiparous mothers (Fig. 3.1C; interaction $f_{2, 553} = 38.369$, $p = <. 0001$). These larger offspring from old mothers were also more resistant to infection regardless of maternal food levels (Fig.3.1D; Maternal age; $\chi^2_2 = 31.05$, $p = <. 0001$ and maternal food; $\chi^2_1 = 0.159$, $p = 0.7$). Maternal age had no effect on offspring reproductive senescence (Appendix A; Fig. 8.1), but reproductive senescence in offspring of normally fed mothers occurred marginally faster than in of offspring of calorie restricted mothers (Appendix A; Fig. 8.1). Maternal treatments had no effect on the rate of offspring mortality or longevity (Appendix A; Fig. 8.2), thus there was no support for the hypothesis that old mothers transmit a senesced state to their offspring (Lansing, 1947, Comfort, 1953, Van Den Heuvel et al., 2016).

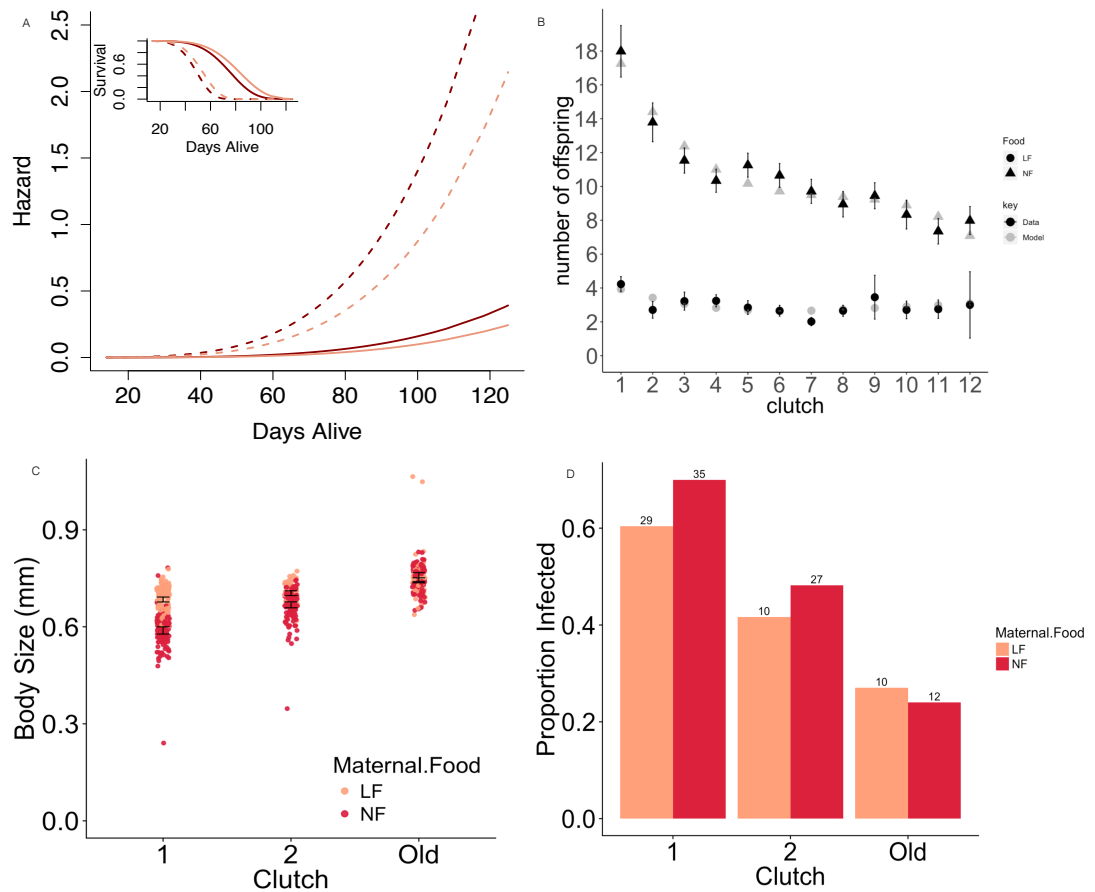


Figure 3.1. Performance measures across two generations. A. Mortality probabilities across four treatments. Compared to uninfected females (solid lines), the rate of mortality was accelerated for infected females (dotted lines) but reduced for both uninfected and infected females when they were kept on a caloric restriction treatment (salmon line) when compared to those on normal food availability (dark red line). Inset: The survival curve of the experimental females showing that calorie restricted individuals lived longer than normally fed individuals and uninfected individuals lived longer than infected individuals. B. The number of offspring produced in clutches 1-13 in the maternal generation from normally fed (NF) females (red line and standard error shading) and low food (LF) females (salmon line and standard error shading). C. Body size measurements taken on the day of birthday from offspring of NF mothers (red dots and standard errors) and LF mothers (salmon dots and standard errors). D. The proportion of infected offspring from each maternal treatment group (NF = red bar; LF = salmon bar).

These results demonstrate that ageing is not characterised by a uniform deterioration in performance across all traits, challenging longstanding predictions of ageing theory. Furthermore, these patterns of juvenescence in offspring quality contrast starkly with observations from humans, where congenital disorders are seen more commonly in offspring of older mothers (Kenny et al., 2013), or with other taxa, where negative impacts of advancing parental age on offspring life-history (Lansing, 1947, Comfort, 1953, Hjelmborg et al., 2015, Benton et al., 2005, Plaistow et al., 2015a) have been recorded. We therefore show that ageing is better studied across generations so as to capture changes in offspring quality, as a more comprehensive assessment of reproductive ageing.

The Effect of Ageing is Environmentally Sensitive

We first model the effect of each ageing trait on R_0 in the absence of the other traits. Under low extrinsic mortality (mild ecological conditions) increasing levels of senescence in age-specific and trans-generational resistance drove an increase in R_0 (Fig 3.2 A-B). In this low extrinsic mortality scenario, old individuals are less likely to die of extrinsic causes, and so populations consist of many old susceptible hosts, driving an increase in transmission. At high extrinsic death rates (harsh ecological conditions) R_0 is decreasing as old susceptible individuals are more likely to have died, leaving a high density of young resistant hosts. Conversely, increasing levels of reproductive and mortality senescence indirectly increase pathogen R_0 under high extrinsic mortality (Fig 3.2 C-D), through their effects on population density and age structure (Fig. 3.2 E-J). Both forms of senescence, under high extrinsic mortality, causes a swell of young individuals, generating a strong supply of potential hosts.

Considering these traits in isolation provides insight into how each measure of ageing can modify pathogen R_0 but the different mechanisms by which they act, and ecological sensitivities of each trait suggest that considering the effects of ageing in single traits is insufficient to understand the complexity of the relationship between ageing and pathogen spread.

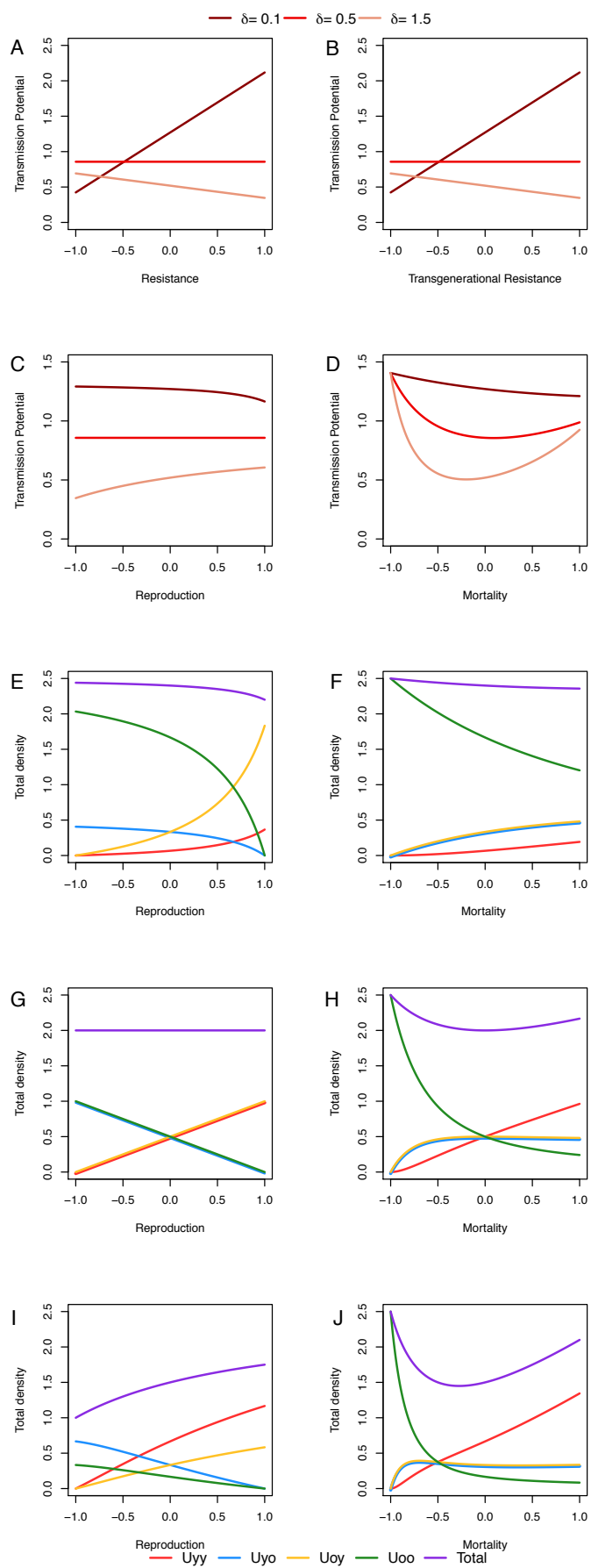


Figure 3.2. The relationship between ageing and transmission potential (R_0) across four traits under variation in extrinsic mortality (δ). Trait ageing spans a spectrum from juvenescence (-1) to senescence (1), representing the range of proportional changes in performance with age. The relationship between A. R_0 and Age specific resistance, B. R_0 and Transgenerational resistance, C. R_0 and Reproduction and D. R_0 and Mortality. Panels E, G, & I display population density with respect to reproductive ageing at low mortality ($\delta < m$; $\delta = 0.1$), medium mortality ($\delta = m$; $\delta = 0.5$) and high mortality ($\delta > m$; $\delta = 1$) respectively. Panels F, H & J display population density and age structure with respect to mortality ageing at low, medium and high extrinsic mortality respectively. Purple represents total population density (N_u), Red; young individuals from young mothers ($U_{Y,Y}$); Blue; young individuals from old mothers ($U_{Y,O}$); Yellow; old individuals from young mothers ($U_{O,Y}$); Green old individuals from old mothers ($U_{O,O}$). Other parameters; $r = k = 2.5$; $c = 0.1$; $m = 0.5$; $\alpha = 1.5$; $\beta = 0.9$

Interacting Traits Result in Dynamic Transmission Landscapes

We next explored the effect of interacting traits on pathogen R_0 , under three ecological conditions as determined by our analytical results (methods). We found that the resulting pathogen R_0 is indeed remarkably dependent on the interactions between different ageing traits and ecological conditions (Fig. 3.3). This is exemplified by the interaction between the two forms of pathogen resistance (Fig. 3.3, A). Under low extrinsic mortality (Fig. 3.3, A.1) juvenescence of resistance has little impact on R_0 alongside juvenescence of trans-generational resistance (a plausible biological pattern that we have observed in *Daphnia*, Fig 3.1 and Garbutt et al (2014a)), but there is a dramatic rise in R_0 when age-specific resistance and trans-generational pathogen resistance are both senescent. However, this ageing pattern in a

high mortality environment results in a reversal of the transmission dynamics (Fig. 3.3, A.3). The interactive effects of reproduction and mortality trajectories on transmission potential show similar ecological dependencies (Fig 3.3, B).

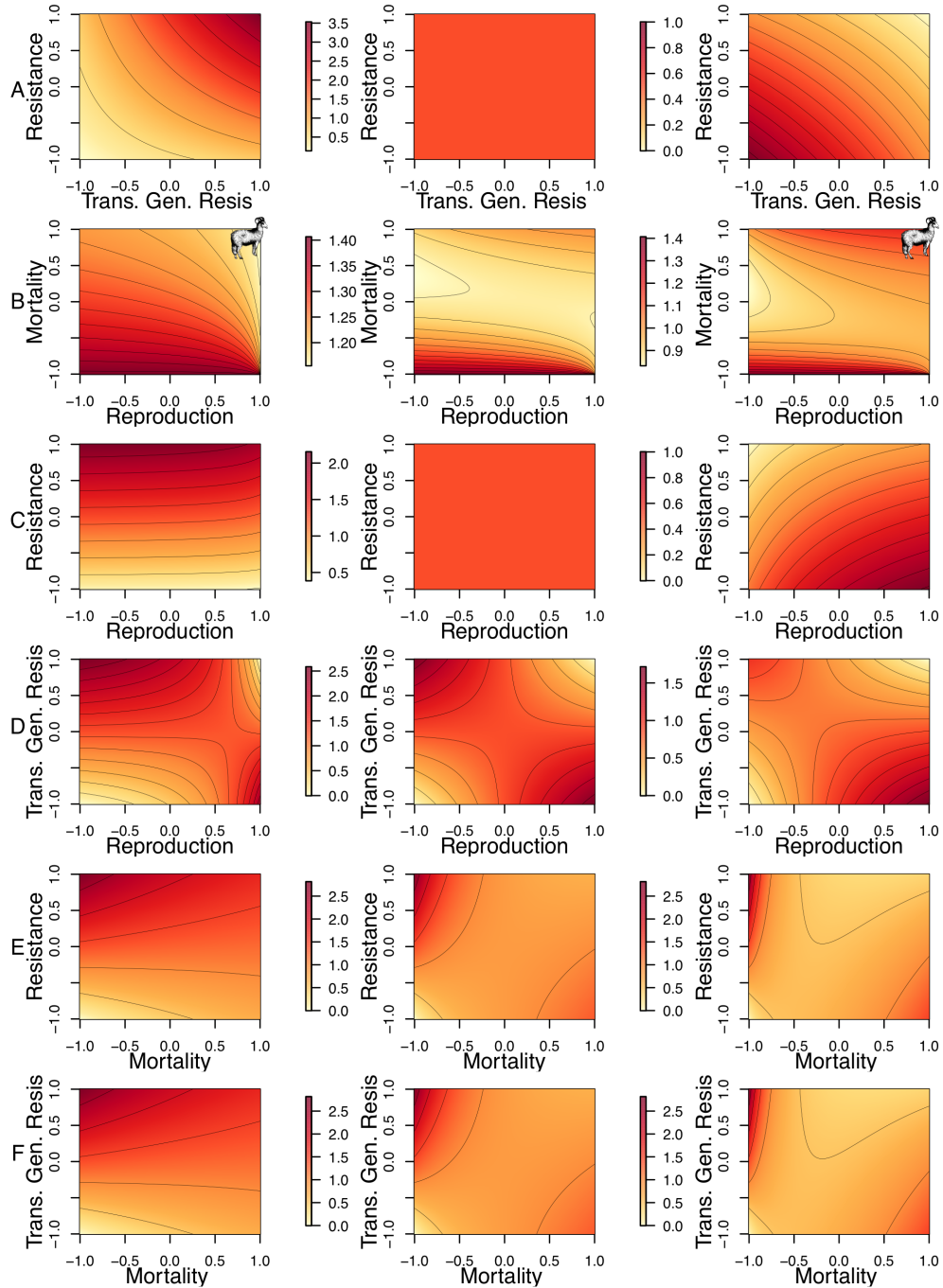


Figure 3.3. The transmission potential landscape as a result of interactions between each pair of ageing traits under three levels of extrinsic mortality, indicative of three different environmental conditions. Colour intensity presents R_0 from darkest red at highest R_0 to white with the lowest R_0 . Numerical R_0 values of each scenario are in the legend. Each axis is the spectrum of ageing from juvenescence (-1) to senescence (1) representing proportional change in each performance measure with age. Column 1 = low extrinsic mortality ($\delta < m$; $\delta = 0.1$), column 2 = medium extrinsic mortality ($\delta = m$; $\delta = 0.5$) and column 3 = high extrinsic mortality ($\delta > m$; $\delta = 1$). In each plot, the two measures of senescence not considered are set to zero. Other parameters; $r = k = 2.5$; $c = 0.1$; $m = 0.5$; $\alpha = 0.9$; $\beta = 0.9$.

These transmission landscapes, and the predictions we might derive from them, are of particular interest for populations subject to rapid ecological change or harvesting, as they may indicate which groups of organisms are at risk of epidemic outbreak within specific ecological conditions. Consider an example mammal, the Soay sheep (Hayward et al., 2015), which shows mortality and reproductive senescence; under low extrinsic mortality, this ageing strategy is not at risk of increasing pathogen transmission (Fig. 3.3, B.1). However, under high extrinsic mortality the typical mammal would be considerably more at risk of epidemic outbreak (Fig. 3.3, B.3) in comparison to other ageing strategies in that environment.

Healthspan Intervention over Lifespan Intervention

Rates of senescence may also differ between populations (Austad, 1993), or even change over relatively short periods of time. For example, there is gathering interest in the development of life extending treatment for humans (Kennedy & Pennypacker, 2015). We applied our model to the human ageing profile (classic senescence in all traits) and considered how the

amelioration of senescence in each trait could impact pathogen transmission. Given that a reduction of extrinsic mortality is in part responsible for the increased lifespan in humans, we assume a low extrinsic mortality scenario. The steady rise in transmission potential as a result of slowing mortality senescence (Fig. 3.4) is particularly striking, but intuitive. By reducing mortality probability, more individuals reach old age (Appendix A; Fig. 8.3A). Without further medical intervention, old individuals are less resistant to infection, so transmission increases. In contrast, interventions that reduce immunosenescence rates would reduce transmission potential by decreasing transmission events (Fig 3.4). However, ageing traits do not occur in isolation, and so the dramatic increase in transmission potential as a result of decreased mortality senescence may be tempered by a reduction in immunosenescence (Fig 3.3, B.1). It is therefore clear, from an epidemiological perspective, that extension of health-span is preferable to lifespan extension as an aim of ageing intervention.

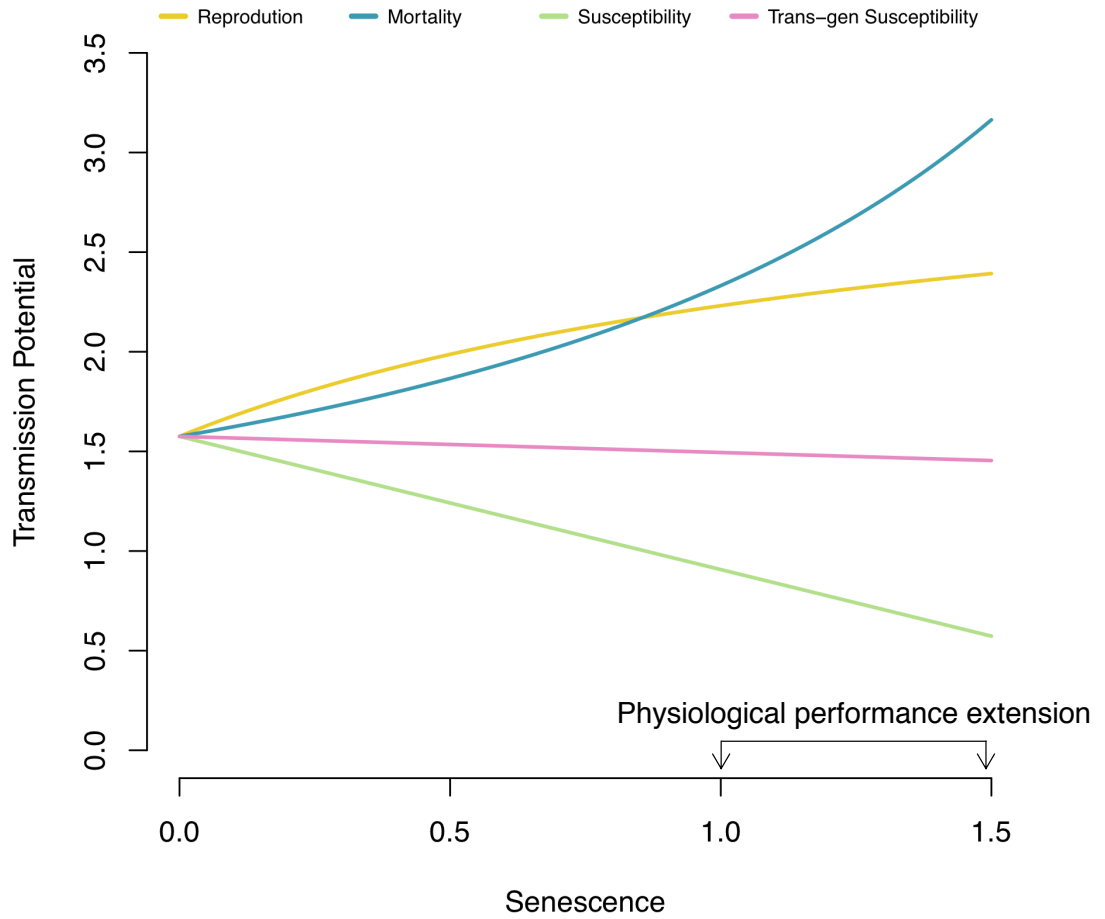


Figure 3.4. The potential impact of senescence manipulation on pathogen transmission potential. We modelled the case where medical intervention increases physiological performance within a population. On the x axis we start at senescence and improve performance, therefore moving towards juvenescence. Yellow; reproductive senescence; Blue; mortality senescence; Green; age specific pathogen resistance; Pink; transgenerational effect of age on resistance. Other parameters; $\delta = 0.1$; $r = k = 2.5$; $c = 0.1$; $m = 0.5$; $\alpha = 1.5$; $\beta = 0.9$ with each remaining ageing trait held constant at 0.5.

We have provided an example of how a single organism can show highly asynchronous ageing patterns, challenging longstanding ageing theory. Our results show that the magnitude, timing and even the direction of ageing may vary across traits in a single organism. Our model shows this individual variation in ageing has substantial consequences at the population level. Future empirical characterisations of ageing must move beyond fecundity and mortality, and theory must strive to accommodate complex patterns of ageing, allowing epidemiological studies to move beyond immune systems or contact rates to consider the effects of all aspects of ageing on infectious disease dynamics.

Experimental Methods

A single clone of *Daphnia magna* was used in all experiments. These were sourced from Kaimes Pond in the south of Scotland. They were acclimated for three generations under controlled conditions in artificial pond medium (33) with 12h L:D at 20°C being fed $\sim 8.75 \times 10^6$ chlorella cells daily. Second-clutch offspring were taken from the third generation to become the maternal generation for the experimental animals.

Maternal Generation

Two offspring were taken from the second clutch of the third generation of acclimated females and were assigned to either normal or low food levels – 1.25abs, as in the acclimating generations or 0.25abs (1.75×10^6) for low food. They were otherwise kept under identical conditions to the acclimated generations. Reproduction and mortality were recorded. For females only providing offspring from clutch one, they were only measured for reproduction once, those providing clutch two, for the first two clutches, and

for those providing offspring from their first clutch after 60 days of age, their total reproduction over this time including this final experimental clutch were recorded.

Analysis

All analysis was carried out using R 3.4.3. The change in reproductive output with age (reproductive senescence) was analysed using a polynomial mixed effect model with a normal distribution (R package lme4 (34)). The number of offspring produced at each reproductive event was the response variable with food (normal N = 72; or low N = 71) as an explanatory factor, and clutch (reproductive events 1-14) as a continuous variable. Clutch was then squared to make the polynomial variable. Clutch and clutch² were both scaled. A unique ID was assigned to each individual and was used as the random effect to account for multiple measures of the same individual. The interactions were sequentially removed by least significance, leaving in lower order interactions if the higher order interaction was significant; all main effects remained in the model and interactions of interest remained in the model. Assessment of autocorrelation was carried out using the R package *nmle*, where the fit of the model with and without an AR1 autocorrelation term for discrete time was compared via maximum likelihood and AIC comparison. The inclusion of the AR1 term did not improve the fit of the model.

Uninfected & Infected Mortality Data

Females were allocated to infection treatment or control groups, and within these dietary restriction or normal food levels (Total N = 223), where they were maintained otherwise as per acclimation. Mortality was modelled with a parametric Accelerated Failure Time model with a Weibull hazard

distribution, as chosen based on AIC comparison (R package flexsurv (35)). When the shape parameter is >1 then the hazard is increasing and when shape = 0 the hazard is constant and thus an exponential model. The response variable was days alive, with food and infection status as explanatory variables. The estimates act upon the scale parameter whilst the shape is held constant(35).

Offspring

From the maternal generation, a subset of mothers from each food treatment contributed offspring from either clutch one, two or the first clutch produced after 60 days of age. Offspring were allocated to the control group or pathogen exposure (Total N = 598). Exposed offspring were dosed with 250,000-spores of *Pasteuria ramosa* directly to the jar on the day of birth. These spores were harvesting from previously infected *D. magna* of the same clone and counted using a Marienfeld Superior Neubauer Improved Haemocytometer. The jar with the new-born and the parasites spores was left unchanged for two days feeding as normal. Infected individuals were culled on day 35 post exposure. Diagnosis was carried out by eye as the carapace of *D. magna* is clear and *P. ramosa* forms a reddish-brown growth visible with the naked eye.

Both control and pathogen exposed individuals had their body size recorded on their day of birth using an Olympus SZX10 and measured using open access software ImageJ. Measurements of the “exposed” treatment group were carried out immediately prior to parasite exposure. Date of birth of each clutch born to the unexposed offspring, and number of individuals in each clutch was recorded.

Analysis

The reproductive analysis was much like the maternal generation with maternal food (normal or low) and maternal age (A, B, or C) as factors, and clutch (1-20). Polynomial variables were not necessary in this analysis. A generalised linear model with binomial error distribution and a logit link function was used to analyse susceptibility using a subset of the data with only the exposed individuals. Body size at birth was analysed with an ANOVA using all offspring. Mortality was analysed using the same approach as the maternal generation but with a Gompertz hazard distribution with two parameters, shape and rate, as chosen by AIC comparison. When the shape parameter is greater than 0 the hazard is increasing and decreasing when < 0 . When $= 0$ the Gompertz model is equal to an exponential distribution with a constant hazard rate. The estimates act upon the rate parameter holding the shape parameter constant(35).

Model Methods

We denote the density of uninfected hosts U and infected hosts I . We consider four age classes with the first subscript denoting an individual's age (Y for young and O for old) and the second, the mothers' age (Y for young and O for old, e.g. $U_{Y,O}$ is an uninfected young host from an old mother). The rate of maturation (moving from age class Y to O) is given by m . We will assume balanced proportional changes in vital rates and pathogen resistance with age, meaning that reductions in a vital rate in old age are balanced by an increase in youth, as assumed in the antagonistic pleiotropy theory of ageing(2). We denote reproduction by young individuals as $r(1 + x)$ and old individuals as $r(1 - x)$ where r is the intrinsic

maximum growth rate and $x \in [-1,1]$ is the proportional change in reproduction with age. Based on our data, we assume that only an individual's age dictates their reproduction and that maternal age has no effect. We similarly denote the mortality rate of young individuals as $d(1 - y)$ and old individuals as $d(1 + y)$ where d is the baseline death rate and $y \in [-1,1]$ is the proportional change in mortality with age. The total population is represented by N with a subscript representing infection status as described above and carrying capacity is denoted by k . The dynamics of each age class in an uninfected, pathogen free population are given by

$$\frac{dU_{Y,Y}}{dt} = \left((r(1+x)U_{Y,Y} + r(1+x)U_{Y,O}) \left(1 - \frac{N_U}{k} \right) \right) - U_{Y,Y}(m + d(1 - y)) \quad 1a$$

$$\frac{dU_{Y,O}}{dt} = \left((r(1-x)U_{O,Y} + r(1-x)U_{O,O}) \left(1 - \frac{N_U}{k} \right) \right) - U_{Y,O}(m + d(1 - y)) \quad 1b$$

$$\frac{dU_{O,Y}}{dt} = mU_{Y,Y} - U_{O,Y}d(1 + y) \quad 1c$$

$$\frac{dU_{O,O}}{dt} = mU_{Y,O} - U_{O,O}d(1 + y) \quad 1d$$

$$N_U = U_{Y,Y} + U_{Y,O} + U_{O,Y} + U_{O,O} \quad 1e$$

By solving for the equilibrium densities of equations 1a-d we get the equilibrium density of each age class

$$U_{Y,Y} = \frac{d^2k(1+x)(1+y)^2(-mr(-1+x) - d(m - r(1+x))(1+y) + d^2(-1+y^2))}{r(d+m+dy)(m-mx+d(1+x)(1+y))^2} \quad 2a$$

$$U_{Y,o} = -\frac{dkm(-1+x)(1+y)(-mr(-1+x) - d(m-r(1+x))(1+y) + d^2(-1+y^2))}{r(d+m+dy)(m-mx+d(1+x)(1+y))^2} \quad 2b$$

$$U_{o,Y} = \frac{dkm(1+x)(1+y)(-mr(-1+x) - d(m-r(1+x))(1+y) + d^2(-1+y^2))}{r(d+m+dy)(m-mx+d(1+x)(1+y))^2} \quad 2c$$

$$U_{o,o} = \frac{km^2(-1+x)(mr(-1+x) + d(m-r(1+x))(1+y) - d^2(-1+y^2))}{r(d+m+dy)(m-mx+d(1+x)(1+y))^2} \quad 2d$$

At this point the total population density (U_N) at equilibrium is given by (Fig.2 E-J).

$$U_N = \frac{k(-mr(-1+x) - d(m-r(1+x))(1+y) + d^2(-1+y^2))}{r(m-mx+d(1+x)(1+y))} \quad 3$$

$$\beta_{Y,Y} = \beta(1-A)(1-M) \quad 4a$$

$$\beta_{Y,o} = \beta(1-A)(1+M) \quad 4b$$

$$\beta_{o,Y} = \beta(1+A)(1-M) \quad 4c$$

$$\beta_{o,o} = \beta(1 + A)(1 + M) \quad 4d$$

We now introduce a pathogen into the population, which transmits at baseline rate β . We assume that host resistance to the pathogen is influenced by age (10-11, 27) and based on our data, maternal age. We denote the proportional effect of age on pathogen resistance as $A \in [-1,1]$, and the effect of maternal age on pathogen resistance as $M \in [-1,1]$. We assume that these effects interact multiplicatively as per an earlier model(5). The transmission to each host age class is then given by

In the presence of the pathogen, virulence v is considered as additional pathogen induced mortality and c is pathogen clearance by the host immune system. For simplicity we assume that there is no change in reproductive output with infection status, resulting in the following population dynamics

$$\frac{dU_{Y,Y}}{dt} = \left((r(1+x)U_{Y,Y} + r(1+x)U_{Y,o}) \left(1 - \frac{N_U}{k} \right) \right) - \beta_{Y,Y}U_{Y,Y}N_I - U_{Y,Y}(m + v + d(1-y)) \quad 5a$$

$$\frac{dU_{Y,o}}{dt} = \left((r(1-x)U_{o,Y} + r(1-x)U_{o,o}) \left(1 - \frac{N_U}{k} \right) \right) - \beta_{Y,o}U_{Y,o}N_I - U_{Y,o}(m + v + d(1-y)) \quad 5b$$

$$\frac{dU_{o,Y}}{dt} = mU_{Y,Y} - \beta_{o,Y}U_{o,Y}N_I - U_{o,Y}(v + d(1+y)) \quad 5c$$

$$\frac{dU_{o,o}}{dt} = mU_{Y,o} - \beta_{o,o}U_{o,o}N_I - U_{o,o}(v + d(1+y)) \quad 5d$$

$$\frac{dI_{Y,Y}}{dt} = \left((r(1+x)I_{Y,Y} + r(1+x)I_{Y,O}) \left(1 - \frac{N_U}{k} \right) \right) - I_{Y,Y}(m + v + c + d(1-y)) \quad 5e$$

$$\frac{dI_{Y,O}}{dt} = \left((r(1-x)I_{O,Y} + r(1-x)I_{O,O}) \left(1 - \frac{N_U}{k} \right) \right) - I_{Y,O}(m + v + c + d(1-y)) \quad 5f$$

$$\frac{dI_{O,Y}}{dt} = mI_{Y,Y} - I_{O,Y}(v + c + d(1+y)) \quad 5g$$

$$\frac{dI_{O,O}}{dt} = mI_{Y,O} - I_{O,O}(v + c + d(1+y)) \quad 5h$$

$$N_I = I_{Y,Y} + I_{Y,O} + I_{O,Y} + I_{O,O} \quad 5i$$

We use the age class equilibria (equations 2a-d) and proportional changes in susceptibility (equations 4a-d) to calculate the pathogen R_0 ,

$$\begin{aligned} R_0 = & (\beta_{Y,Y}U_{Y,Y} + \beta_{O,Y}U_{O,Y} + \beta_{Y,O}U_{Y,O} + \beta_{O,O}U_{O,O}) \left(\frac{U_{Y,Y}}{U_N} * \frac{1}{m + v + c + d(1-y)} \right. \\ & + \left(\frac{U_{O,Y}}{U_N} + \frac{U_{Y,Y}}{U_N} * \frac{m}{m + v + c + d(1-y)} \right) * \frac{1}{v + c + d(1+y)} + \frac{U_{Y,O}}{U_N} \\ & * \frac{1}{m + v + c + d(1-y)} + \left(\frac{U_{O,O}}{U_N} + \frac{U_{Y,O}}{U_N} * \frac{m}{m + v + c + d(1-y)} \right) \\ & \left. * \frac{1}{v + c + d(1+y)} \right) \end{aligned} \quad 6$$

where transmission is weighted by the length of time spent in each age class, and in the uninfected old age class transmission is weighted by the probability that individuals in the corresponding uninfected young age class will successfully mature before an infection is cleared.

We will restrict our analytical assessment of the effects of ageing to the initial evolution of senescence or juvenescence in the trait, i.e. by evaluating effects at the point $x = 0$, $y = 0$, $A = 0$, $M = 0$ (though our numerical analysis explores the full spectrum). Age-related changes in reproduction and mortality have the potential to directly affect population density and age-structure. The change in population density with respect to x is given by

$$\left. \frac{dU_N}{dx} \right|_{x=0, y=0, A=0, M=0} = \frac{dk(d - m)}{r(m + d)} \quad 7$$

This is positive so long as $d > m$. When death exceeds maturation, the population is predominantly young. Under our pleiotropic model of ageing, young hosts reproduce in greater quantities than old hosts, maintaining increases in population density in the presence of reproductive senescence. At $x = 0$, $y = 0$, $A = 0$, $M = 0$, the change in density with respect to y is given by

$$\left. \frac{dU_N}{dy} \right|_{x=0, y=0, A=0, M=0} = \frac{dk(d - m)}{r(m + d)} \quad 8$$

$\frac{dU_N}{dy} > 0$ so long as $d > m$. When the rate of mortality exceeds the rate of maturation most hosts are young, thus increased mortality rate in old age, and decreased mortality rate in young age, increasing population density.

We now evaluate the effects of ageing in all four traits on pathogen R_0 , again at the point of their initial evolution, $x = 0$, $y = 0$, $A = 0$, $M = 0$. The effect of senescence in reproductive rate on pathogen R_0 is given by

$$\left. \frac{dR_0}{dx} \right|_{x=0, y=0, A=0, M=0} = \frac{Bdk(d-m)}{(d+m)r(c+d+v)} \quad 9$$

which is positive whenever $d > m$. This mirrors the condition for reproductive senescence to increase total population density, meaning that the primary effect of reproductive senescence on pathogen spread, in the absence of age-related changes in other traits, is owing to its effects on population density.

The effect of mortality senescence on pathogen R_0 is positive so long as

$$d > \frac{1}{2} \left(-c - v + \frac{2m(c+r+v)}{c+2m+r+v} + \sqrt{c^2 + 2cv + v^2 + \frac{8m^2(c+r+v)(c+m+r+v)}{(c+2m+r+v)^2}} \right) \quad 10$$

i.e. the effect is positive so long as the mortality rate is sufficiently high. The complexity of the condition under which mortality senescence increases pathogen R_0 is justified by the three-way relationship mortality senescence has with pathogen R_0 . As we have shown, mortality senescence affects population density and age structure, and further to this it will influence the duration of infection.

The effect on R_0 of the proportional change to resistance with age is given by

$$\left. \frac{dR_0}{dA} \right|_{x=0, y=0, A=0, M=0} = \frac{Bk(d-m)(d-r)}{(d+m)r(c+d+v)} \quad 11$$

which is positive when $d < m$. When the rate of maturation exceeds the rate of mortality, most of the population would be old. Thus, increasing susceptibility with age will increase pathogen transmission.

Finally, the effect of trans-generationally derived susceptibility on pathogen R_0 is also given by

$$\left. \frac{dR_0}{dA} \right|_{x=0, y=0, A=0, M=0} = \frac{Bk(d-m)(d-r)}{(d+m)r(c+d+v)} \quad 12$$

which is again positive so long as $d < m$. When the rate of maturation exceeds the rate of mortality, the population is comprised mostly of offspring from old individuals. Thus, increased susceptibility in the offspring of old mothers increases pathogen R_0 .

Chapter 4 Between and Within Population Genetic Variation in Life History Traits in *Daphnia magna*

Summary

There is considerable genetic variation in longevity both between and within populations, though it is expected that there would be more variation between populations than within. Antagonistic pleiotropy is proposed as a mechanism for the maintenance of this variation, which may be detected as genetic trade-offs in life history performance. Using the freshwater crustacean *Daphnia magna*, we show that there is a comparable level of genetic variation within a population as is found between populations in longevity and the response to dietary restriction. Genetic trade-offs in 49 genotypes from the same population were also studied but no evidence of antagonistic pleiotropy was found. We further tested the relationship between life-history traits and longevity using an alternative experimental and statistical approach. Despite increased statistical power this second approach also revealed no genetic relationship between longevity and life-history performance in *Daphnia magna*.

Introduction

As components of fitness, life history traits are thought to be under stabilizing selection, which should reduce genetic variation (Barton & Keightley, 2002). However, there is extensive variation, both within and between populations in the expression of life history traits (Barton & Keightley, 2002). There are numerous proposed mechanisms by which selection may directly and indirectly maintain genetic variation. Selection may act directly upon a trait through heterozygote advantage or frequency

dependent selection (Barton, 1990, Christie et al., 2018, Barton & Keightley, 2002). Alternatively, mutation-selection balance can maintain genetic variation as deleterious alleles are eliminated by selection at a rate analogous to their appearance. It is proposed that populations in mutation-selection balance will have a higher frequency of deleterious alleles that are expressed later in life than early acting deleterious alleles (Clark, 1994). This idea is the core of the mutation-accumulation theory (Medawar, 1952) which, along with the antagonistic pleiotropy model of ageing (Rose, 1982, Williams, 1957), seeks to explain both the maintenance of variation in life history traits (Anderson et al., 2011, Arbuthnott, 2018, Brown & Kelly, 2018, Carter & Nguyen, 2011, Rodriguez et al., 2017, Zajitschek & Connallon, 2018) and the evolution of senescence.

Senescence precedes the end to an individual's lifespan and is characterised as a period of physiological decline with an increasing probability of mortality. One of the main causes for this decline is thought to be the weakening of selection with an increase in age. Once an individual has reproduced, deleterious alleles have been passed on, and thus selection is inefficient at removing them from a population (Haldane, 1941, Charlesworth, 2000, Hamilton, 1966), resulting in a selection shadow (Fabian & Flatt, 2011, Flatt & Partridge, 2018). The weakening of selection with age accounts for the amassed deleterious mutations proposed under both mutation-accumulation and antagonistic pleiotropy, with a key difference between the two theories. Whilst mutation – accumulation proposes deleterious alleles accumulate passively, under antagonistic pleiotropy, it is proposed that alleles conferring a fitness benefit early in life are actively selected despite being associated with late acting deleterious effects. Antagonistic pleiotropy therefore should manifest as life history

trade-offs, for example between early and late life reproduction or sexual and non-sexual traits, such as reproduction and immune investment (Arbuthnott, 2018).

Lifespan is a fundamental life history trait with substantial variation seen across species, as well as between and within populations of the same species. Through quantitative trait analysis (Mackay, 2002, Leips & Mackay, 2000, Paaby & Schmidt, 2009), gene deletion (Campos et al., 2018) and gene silencing (Guarente & Kenyon, 2000), considerable progress has been made towards understanding the genetics of lifespan. These studies suggest that variation in lifespan is under a degree of genetic control, with heritability between 10-30% (Mackay, 2002). Of particular interest is the relationship between the insulin/IGF signalling pathway and longevity (Kenyon, 2010, Altintas et al., 2016, Partridge & Gems, 2002, Gems & Partridge, 2013), where single mutations for genes encoding the pathway can double lifespan in *C. elegans* (Kenyon et al., 1993), whilst extended lifespan and delaying the onset of physiological decline in *D. melanogaster* (Partridge et al., 2011, Wessells et al., 2004) and mice (Selman et al., 2008). The lifespan impacts of the insulin/IGF signalling pathway are thought to have evolved due to antagonistic pleiotropy as evidenced by the sterility of some insulin/IGF signalling pathway mutant *D. melanogaster*, where the benefit of shortened lifespan may be borne through increased early life reproduction (Partridge & Gems, 2002).

The insulin/IGF signalling pathway, along with the target of rapamycin (TOR) network, is part of a signalling system that links nutrient consuming processes (like reproduction and growth) to the nutrient status of the animal. Dietary restriction (DR) has become a regularly used nonpharmacological

intervention that manipulates the insulin/IGF signalling and TOR pathways resulting in the extension of lifespan. The use of the gerontological paradigm, whereby an organism's longevity is extended by the modest restriction of nutrient intake above that of malnutrition, in exchange for reduction in life time reproductive success, was first observed in rats, but later in spiders, rotifers, and model organisms such as *C. elegans*, and *Daphnia* spp. (Austad, 1989, Kim et al., 2014, Klass, 1977, Latta et al., 2011, Lynch & Ennis, 1983, McCay et al., 1935, Sawada & Carlson, 1987). The application of DR also leads to increased resistance to certain stressors and the amelioration of age-related pathologies such as metabolic breakdowns, reduced inflammatory responses, protection from brain deterioration, and reductions in cases of sarcopenia, cardiovascular disease and endometriosis (Anderson & Weindruch, 2012, Colman et al., 2009, Gems & Partridge, 2013, Kemnitz, 2011).

Further to trade-offs between reproduction and lifespan, DR and other similar manipulations of the insulin pathways highlight the role of the immune system in determining longevity. Work on *Drosophila* and mice have shown that DR results in a reduction or delay in immune gene expression in exchange for increased longevity (Lee et al., 1999, DeVeale et al., 2004, Pletcher et al., 2002, Seroude et al., 2002), suggesting the immune system may be a "necessary evil" resulting in a trade-off that ensures early life survival and therefore fitness gains (DeVeale et al., 2004). The effect of DR however, is not universal, and work utilising lines from the *Drosophila* Genetic Reference Panel (DGRP) collection has shown that lines emulating the level of genetic variation expected in wild populations differ significantly in their longevity and response to dietary restriction (Dick et al., 2011). The level of genetic variation within wild populations of *D. melanogaster* has also

been explored by sampling directly from the field (Schmidt et al., 2005, Paaby & Schmidt, 2009), but few studies have compared the genetic variation in lifespan or the response to dietary restriction within populations to that between populations (Paaby & Schmidt, 2009).

Utilising the crustacean *Daphnia magna*, we conducted a series of experiments to characterise the genetic variation in longevity and life history performance. We first sought to compare the genetic variation in longevity and the response to dietary restriction in genotypes from different populations, to that in genotypes from the same population. Genetic variation between populations is expected to be greater than within a population, because those within populations will be genetically more similar and experience the same environmental selection. We then sought to characterise genetic variation in longevity within a single population more deeply. In an experiment that included 49 clonally replicated genotypes, we measured longevity and life history traits that might covary with longevity under the antagonistic pleiotropy hypothesis. If antagonistic pleiotropy could be detected through life history trade-offs in this system, it may also suggest that the effect of dietary restriction is indeed a consequence of antagonistic pleiotropy as suggested by Partridge & Gems (2002). We also consider an alternative statistical approach to detect trade-offs between life history traits. By determining the ten longest and ten shortest-lived genotypes from the 49 within population genotypes used, we produced two groups with significantly different lifespans. We then asked if longevity “group” can explain life history performance or susceptibility to pathogenic infection. Extensive power analysis indicated that this second approach would provide more statistical power than the classic correlational approach.

Methods

Study System

This series of experiments was conducted using the freshwater invertebrate crustacean *Daphnia magna* and its natural parasite *Pasteuria ramosa*.

Daphnia magna reproduces parthenogenically, producing genotypically identical daughters. *Pasteuria ramosa* is a spore forming bacteria that is transmitted environmentally, when infected hosts die, releasing the spores into water and sediment, where they are ingested by filter feeding hosts. Diagnosis of infection is done by eye due to castration of the host and obvious reddish growth within the body cavity visible through the transparent carapace as the bacteria grows.

Acclimation

Prior to the experiments, replicates of the *Daphnia* genotypes were put through three generations of acclimation. This harmonises environmental effects arising from variation in stock conditions. During this period, each individual was maintained in a 60ml glass jar filled with artificial pond medium (Kluttgen et al., 1994). This was changed twice weekly and when offspring were produced. Each individual was fed 1.25 absorbency (7×10^6) *Chlorella vulgaris* cells daily and was maintained on a 12:12 L:D cycle at 20°C. Offspring from the second clutch initiated each generation, including the experimental generation.

Experiment 1: Between Population Variation in Longevity & the Response to Dietary Restriction

This experiment used eight genotypes from geographically dispersed populations. These were obtained from the *Daphnia magna* diversity panel

held in Basel, Switzerland

(<http://evolution.unibas.ch/ebert/research/referencepanel/>). They are [clone ID (Country of Origin)]: BEK22 (Belgium), Clone 32 (UK), FIFAV1 (Finland), GBEL75 (UK), GG8 (Germany), ILPS1 (Italy), MNDM1 (Mongolia), RUYAK1-6 (Russia). From the acclimated females of all eight genotypes, two offspring from clutch 2 were taken. One was assigned to ad libitum food (AL: 1.25abs as per acclimation) and one was assigned to a dietary restriction (DR: 0.25 abs) treatment. Each food treatment and genotype combination were replicated 24 times. Date of birth and date of death were recorded. Other conditions were identical to the acclimation period.

Experimental Analysis

A cox proportional hazards model was used to test for differences in longevity between the two food treatments for all eight genotypes. This was done using the Survival package in R (R core team 2017). The response variable was days alive, with genotype, food treatment and their interaction, as fixed effects. There was no census as all individuals were followed from their day of birth to the day of death.

Experiment 2: Within Population Genotypic Variation in Longevity & the Response to Dietary Restriction

This experiment used eight randomly chosen genotypes originally collected from Kaimes pond in the south of Scotland. The experimental procedure and analysis were identical to experiment 1.

Experiment 3: Genotypic Variation in the Relationship Between Reproduction and Longevity

The goal of this experiment was to use a larger number of *D. magna* genotypes to study relationships between longevity and other life history traits. Prior to the start of this experiment two power analyses were run. The power analyses were put through 1000 iterations and were parameterised using the means, standard deviations and error standard deviations from two data sets. The longevity data came from the control individuals in experiment 1, and the reproductive data came from a pilot study using the same eight genotypes. The measures of early reproduction were the number of offspring produced at the first reproductive event, and the age at first reproduction.

Power Analyses

The first power analysis was used to determine the experiment size (how many genotypes and replicates within each treatment) necessary to detect genotypic correlations between longevity and the two measures of reproductive performance. From this it was determined that the power to detect the genotypic relationship between reproductive characteristics and longevity was low even when using an excessive number of genotypes and replicates of each genotype (Table 4.1).

Table 4.1. The proportion out of 1000 power analysis runs that a significant correlation was found between longevity (days alive) and early reproductive performance measures, for simulated experiments of 12, 18, 24 or 48 replicates of 8, 20, 30, 50, 70 or 100 clones. Parameter estimates used in the power analyses were from pilot data and previous experiments as stated in chapter methods.

Days Alive x Age at 1 st Reproduction						
Replicates	Number of Clones					
	8	20	30	50	70	100
12	0.056	0.049	0.048	0.043	0.050	0.051
18	0.054	0.056	0.076	0.067	0.047	0.063
24	0.060	0.041	0.043	0.049	0.052	0.052
48	0.042	0.045	0.049	0.058	0.049	0.053
Days Alive x Early Reproduction						
12	0.058	0.042	0.062	0.040	0.042	0.055
18	0.058	0.055	0.050	0.048	0.056	0.041
24	0.050	0.041	0.056	0.045	0.056	0.052
28	0.046	0.042	0.050	0.053	0.051	0.047

The second power analysis was therefore used to simulate an alternative experimental design and statistical approach to investigate relationships between longevity and life history traits. Using this power analysis, we determined how many genotypes and replicates would be necessary to produce groups of genotypes that were “long lived” and “short lived”. It was determined that 49 genotypes with 18 replicates would be suitable to identify two groups, each consisting of 10 genotypes each, one long lived, the other short-lived (Fig. 4.1; $t = -264.9$, $p = <0.0001$)

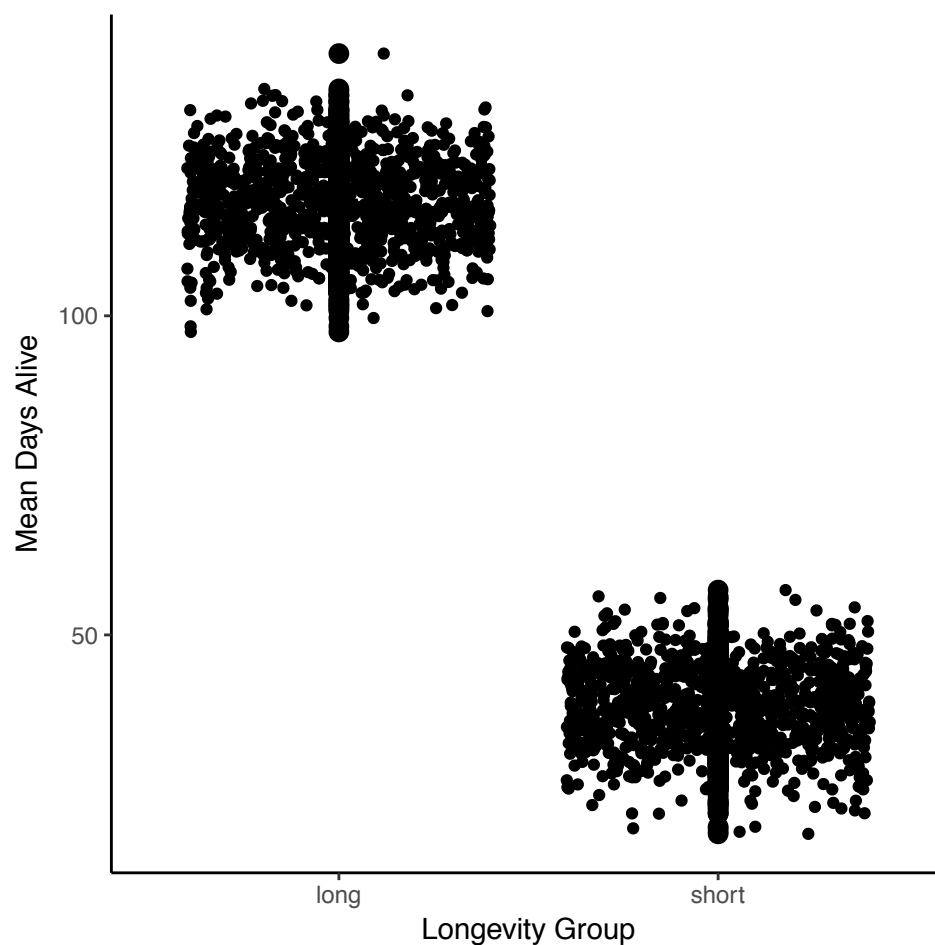


Figure 4.1. The results of a power analysis that simulated data 1000 times to show that using 49 genotypes, two groups containing 10 genotypes each, that differ significantly in their longevity could be produced.

Based on the results showing similar effect sizes in experiments 1 & 2, and owing to the results of the power analyses, in the third experiment 49 genotypes were chosen at random, from the Kaimes population. Eighteen replicates of each genotype were used, and data were collected on reproduction and longevity. Previous measurements of age-specific reproduction (chapters 2 & 3) have shown little variation around the mean resulting in pleasing confidence intervals. In the interest of time and accuracy we there collected data from, nine of the 18 replicates on age at first and ninth reproduction and the number of offspring produced in these clutches. This provided data on age at first reproduction, the number of offspring produced, and late reproduction ("late" as determined by data in chapter 3) from which reproductive senescence could be estimated.

Experimental Analysis

Pearson correlations were used to analyse genetic correlations between reproductive measures and longevity. A mean for each clone was used meaning there were 49 data points for each correlation. The variables were: longevity, number of offspring at clutch one, and the slope of the difference in reproductive output between clutch one and clutch nine.

The data was then split by identifying the top 10 longest and 10 shortest living genotypes, to produce a two-level categorical explanatory variable ("group"). A t-test was used to confirm differences in lifespan between the long-lived and short-lived groups. The relationship between longevity and reproduction was analysed again this time using the measures of reproduction as response variables, group as a fixed explanatory variable and clone as a random effect using the lme4 package in R (Bates et al., 2014)

Experiment 4: Genotypic Variation in Susceptibility & Longevity

In this experiment 24 replicates of the 10 long-lived and 24 of the 10 short-lived genotypes determined in experiment 3 were exposed to *P. ramosa*. On the day they were born, their body size was measured, and they were then given 200,000 spores, applied directly to the jar. They were maintained as per acclimation for 28 days on ad libitum food. Diagnosis was made as soon as infection was clearly visible, and infected individuals were culled on day 28 post infection.

Analysis

Genetic variation in susceptibility was analysed using a generalized linear model (link = logit) with longevity group as a fixed explanatory variable and clone as a random effect. The response variable was a binomial y/n whether an individual became infected or not using lme4 in R (Bates et al., 2014)

Results

Experiment 1: Between Population Variation in Longevity & the Response to Dietary Restriction.

When using genotypes from different global locations, there was a significant interaction between food and genotype for differences in longevity in response to DR (Fig. 4.2; $\chi^2 = 27.69$, Table 4.2; $p = 0.0002$).

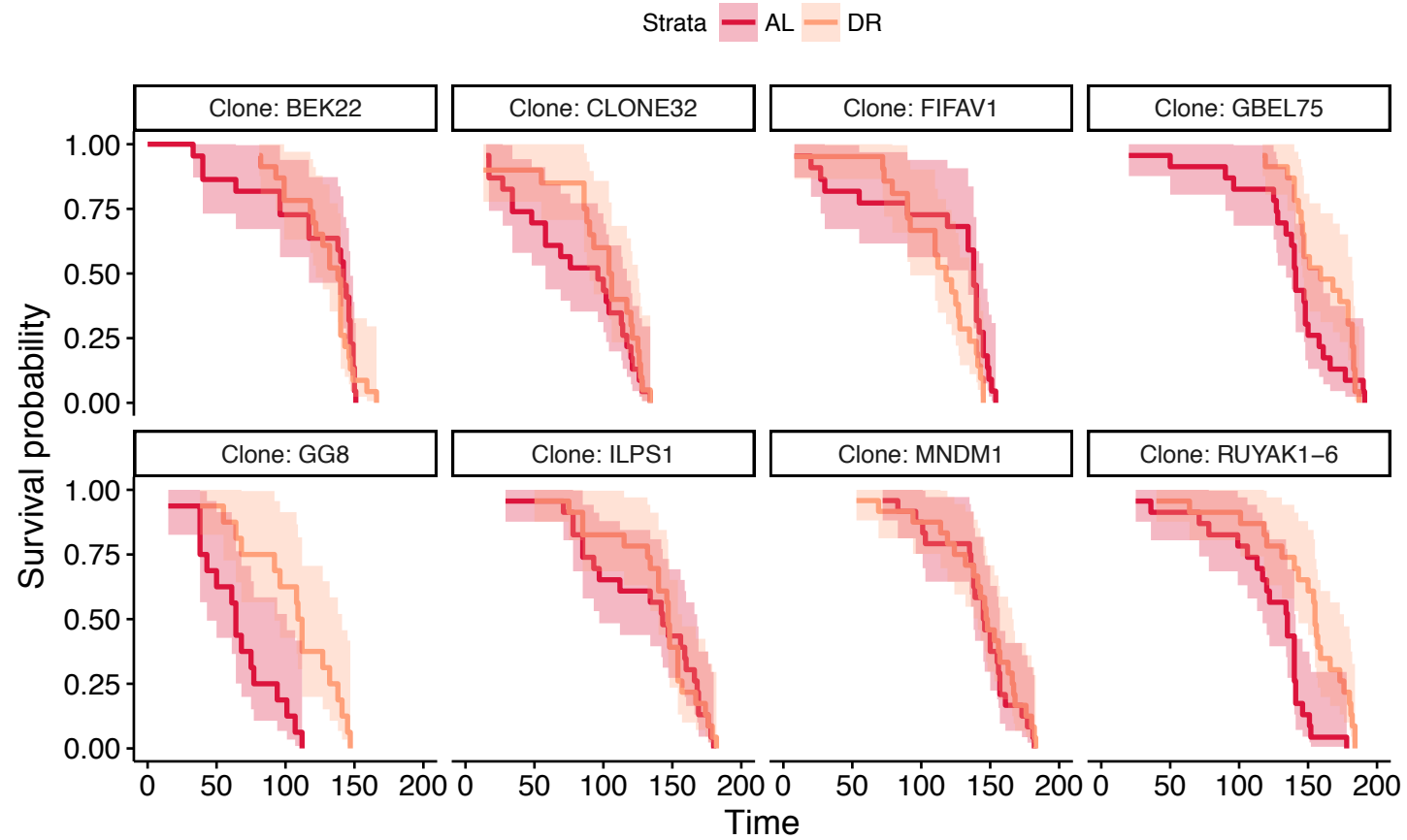


Figure 4.2. The longevity and survival probabilities in *D. magna* from eight geographically isolated populations, kept on Ad Libitum (AL; red) or Dietary Restriction (DR; orange) food treatments.

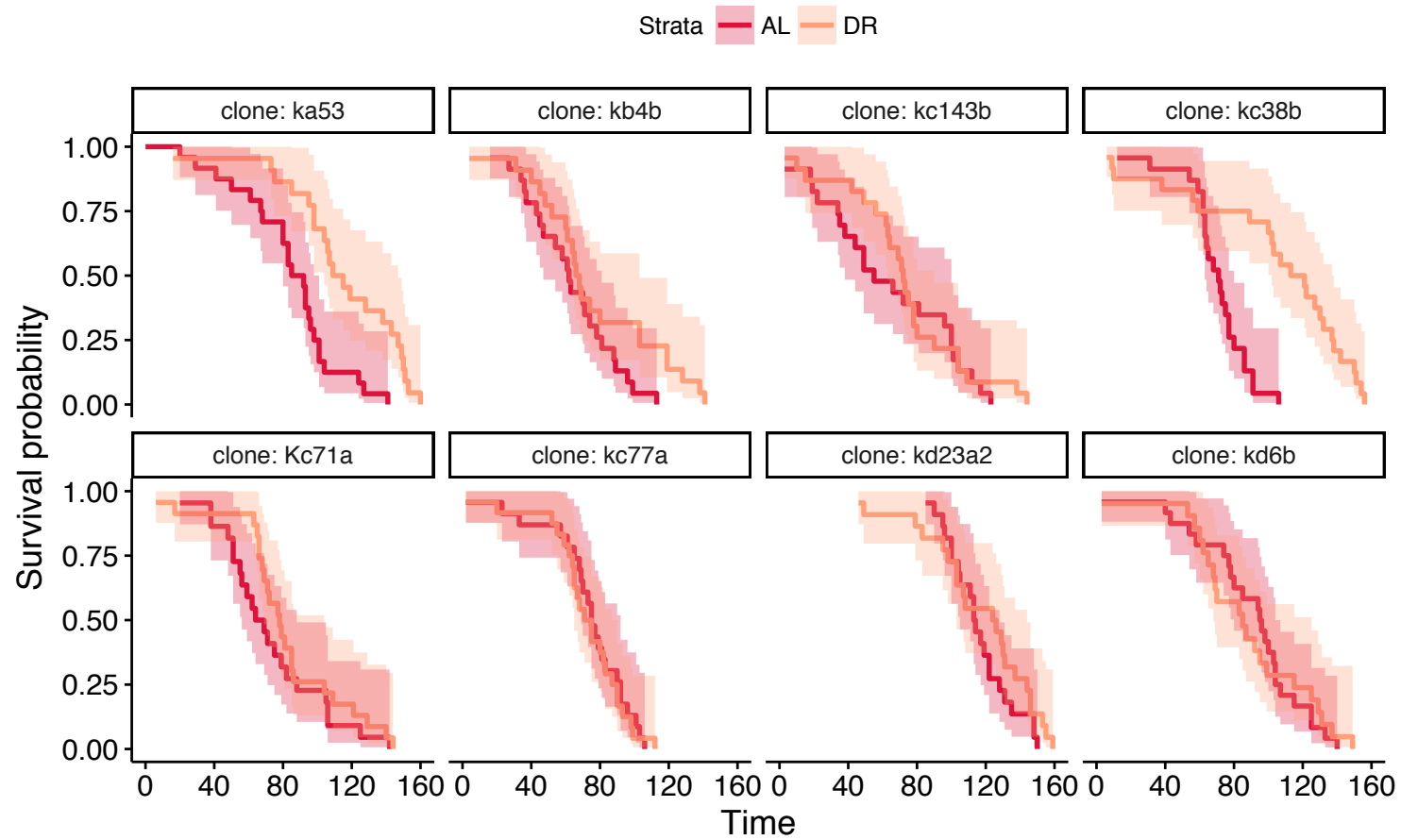


Figure 4.3. The longevity and survival probabilities of each Kaimes clone at each time step (age in days) for Ad Libitum (AL; red) fed and Dietary Restricted (DR; orange) treatment groups.

Table 4.2. Model results for the response to dietary restriction seen in experiment 1, using within population genotypes, and experiment 2, between population genotypes.

Within Population				
Variable	Loglik	χ^2	<i>df</i>	<i>p</i>
Null	-1792.3			
Food	-1783.3	18.1	1	<0.001
Clone	-1746.7	73.3	7	<0.001
Interaction	-1732.7	27.9	7	<0.001
Between population				
Variable	Loglik	χ^2	<i>df</i>	<i>p</i>
Null	-1698.3			
Food	-1694.7	7.2	1	0.007
Clone	-1622	145.4	7	<0.001
Interaction	-1608.1	27.7	7	<0.001

Experiment 2: Within Population Genotypic Variation in Longevity & the Response to Dietary Restriction

For the eight genotypes from one population, there was a significant interaction between genotype and food treatment for differences in longevity in response to DR (Fig. 4.3; $\chi^2 = 27.932$, Table 4.2; $p = 0.0002$).

Experiment 3: Genotypic Variation in Reproduction & Longevity

There was no significant genetic correlation between reproductive senescence and longevity (Fig. 4.4A; $r = -0.17$, $p = 0.2542$) or the number of offspring produced in the first clutch, and longevity (Fig. 4.4B; $r = 0.1$; $p = 0.4781$). There was also no correlation between the age at first reproduction and longevity (Fig. 4.4C; $r = -0.17$, $p = 0.2547$).

The long lived and short-lived groups were made up of ten genotypes each and had significantly different lifespans (Fig. 4.5; $t_{15.74} = 11.419$, $p < 0.0001$). Using “group” as an explanatory variable and the reproductive measures as response variables, there was no difference between long lived and short-lived groups in their reproductive senescence (Fig. 4.6A; $f = 0.013$, $p = 0.91$) and there was no difference between the long and short-lived groups in the number of offspring, or age at first clutch (Fig 4.6B; $f = 0.333$, $p = 0.571$ & Fig. 4.6C; $f = 0.114$, $p = 0.74$ respectively).

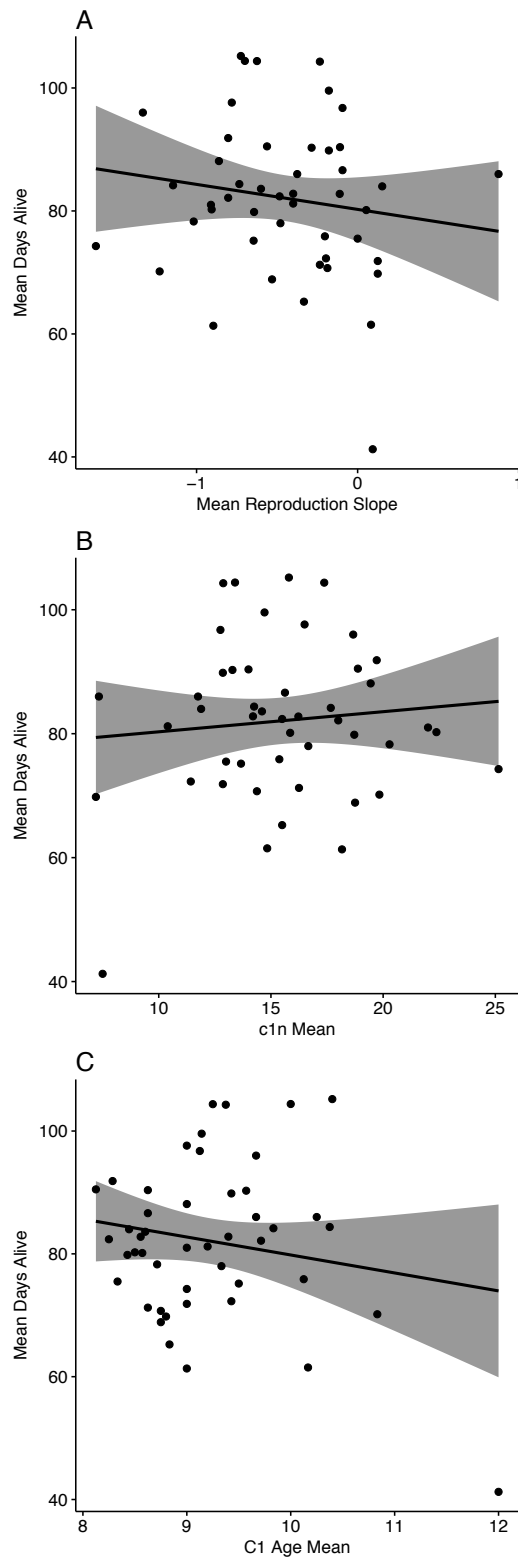


Figure 4.4. The correlation between A. Mean number of days alive per genotype, and the mean slope of reproductive senescence for each genotype. B. Mean number of days alive per genotype, and the number of

offspring produced in the first clutch. C. Mean number of days alive per genotype, and the mean age at first reproduction for each genotype.

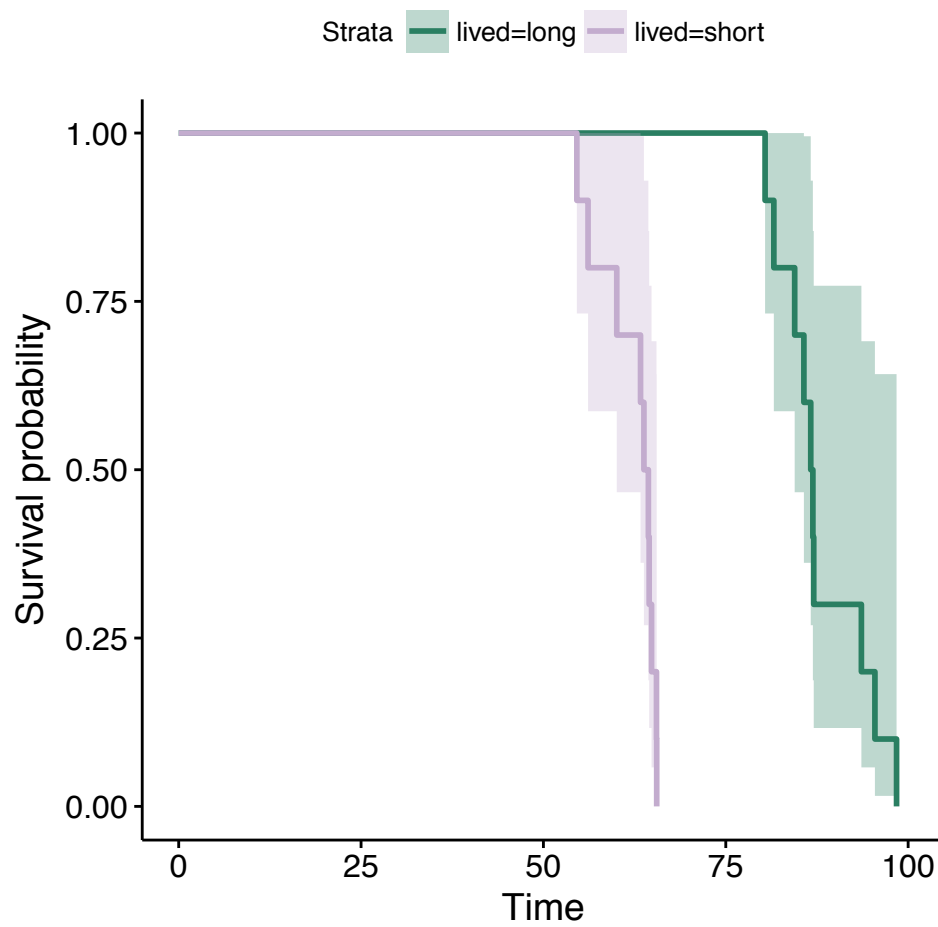


Figure 4.5. The long-lived group (green) live significantly longer than the short-lived group (purple).

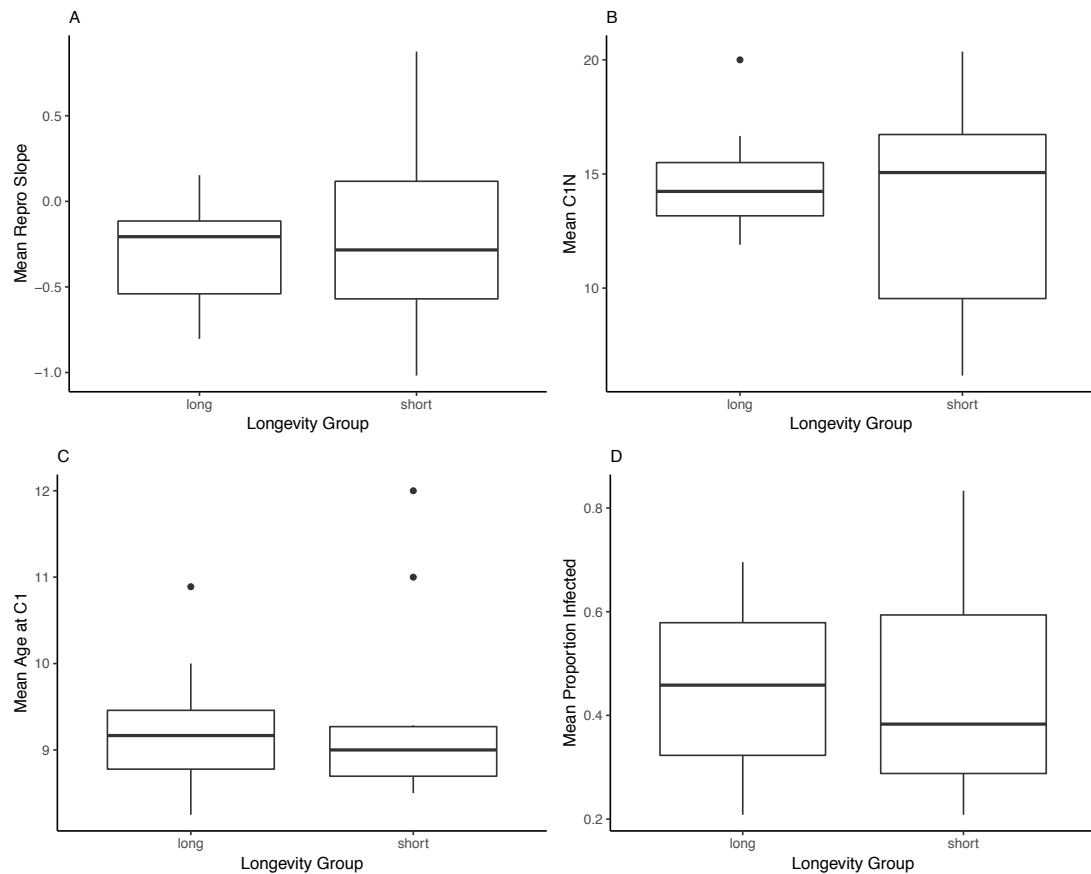


Figure 4.6. A. There was no difference between long- and short-lived groups in their reproductive senescence B. There was no difference between long lived and short-lived groups in the number of offspring they produced in their first clutch. C. There was no difference between the long lived and short-lived groups and the mean age at first reproduction. D. There was no difference between long- and short-lived groups in their susceptibility to infection

Experiment 4: Longevity & Susceptibility

There was no relationship between longevity group and susceptibility to infection (Fig. 4.6D; $f=0.038$, $p=0.844$).

Discussion

Genetic differences between populations are generally expected to be larger than the variation within populations, simply because environmental factors, like rates of extrinsic mortality (Pietrzak et al., 2015, Williams, 1957) vary in space. However, we observed similar effect sizes for genetic variation in response to DR in genotypes originating from the same population, to those from different populations (Table 4.2). An explanation for this may be that *D. magna* populations are spatially variable, such that local adaptation on a fine scale to one area of the population maintains within population genetic variation. This suggests that genotypes in one locale have higher fitness there than elsewhere. In the case of the between and within population experiments, the application of DR, which changes the environment an individual experiences, highlights these genotype-by-environment interactions.

Polymorphisms can also be maintained under pathogen induced negative frequency dependent selection (Decaestecker et al., 2007). Parasite-host coevolution requires genetic variation in host susceptibility for natural selection to act upon. There are many natural pathogens of *Daphnia* including haplosporidia, microsporidia, bacteria, fungi and at least one virus (Toenshoff et al., 2018, Little & Ebert, 1999), and indeed, considerable variation in susceptibility is seen. Many populations are infected with more than one type of pathogen at a time (Little & Ebert, 1999), with no one host genotype showing resistance or susceptibility to all pathogens, or even all variants of the same pathogen species (Decaestecker et al., 2003, Carius et al., 2001, Haag & Ebert, 2004). Populations infected with multiple pathogens also show significant changes in genotype frequency (Haag & Ebert, 2004),

suggesting that selection against susceptibility to a particular pathogen is common.

Trade-offs are a widely cited explanation for the evolution of senescence. In our experiments on *D. magna*, neither lifespan nor senescence were correlated with other life history traits. An explanation for this may be that there is a phenotypic rather than genetic trade-off occurring. The Disposable Soma theory of ageing predicts that the investment of limited resources into reproduction or early life fitness traits will result in reduced late-life fitness measures such as reproduction and longevity. Rather than being due to pleiotropy however, it is predicted to be the result of amassed errors in somatic cellular copying mechanisms (Kirkwood, 1977). The discrepancy in resource allocation should therefore result in a detectable phenotypic trade-off between survival or other measures of somatic investment, such as growth, and reproduction. Though this prediction of detectable age-specific trade-offs is synonymous between the two theories, the Disposable Soma theory is framed by optimisation theory and physiology (Partridge & Barton, 1993). Body size may be a more direct measure of somatic investment, and as such the inclusion of body size as a potential correlate could have yielded more insight into the allocation of resources to somatic maintenance and life history performance. In the context of dietary restriction, an investment of resources into survival through investment in cellular maintenance, rather than reproduction may explain an observed extension of lifespan. This is supported by the evidence of increased levels of apoptosis and cellular maintenance in dietary restricted individuals (Dunn et al., 1997, Wachsman, 1996). As evidenced by the within and between population experiments however, dietary restriction does not extend lifespan in all genotypes, and thus the

empirical support for resource allocation in the context of DR is somewhat mixed.

Although there is evidence for trade-offs in a wide range of organisms (Rose & Charlesworth, 1981, Rose, 1984b, Partridge & Fowler, 1992, Travers et al., 2015, Carranza et al., 2004, Lemaître et al., 2015, Thomas et al., 2000), positive correlations, or no correlation, between traits may be as common as negative ones (Réale & Festa-Bianchet, 2000, Lemaître et al., 2015, Spitze et al., 1991, Reznick et al., 2000, Messina & Fry, 2003, Vorburget, 2005). It is often assumed that the statistical or observed correlation accurately reflects the functional trade-off underlying it, but this is not always the case. As explained by the “Y-model” of resource acquisition and allocation (van Noordwijk & de Jong, 1986, Roff & Fairbairn, 2007), positive statistical relationships may be found where negative functional relationships are expected, or even exist, due to the variance in resource acquisition in relation to the variance in resource allocation. Where the variance in acquisition is greater than the variance in allocation the relationship between two traits will appear to be positive. Roff & Fairbairn (2007) provide a detailed discussion on the importance of the relative mean sizes of the coefficient values in determining the sign of the covariance also.

Positive genotypic trade-offs may also manifest when multiple loci are involved in acquiring limiting resources (Houle, 1991). Similarly, positive phenotypic trade-offs may be detected when many traits are involved in the functional trade-off, such that when testing for trade-offs between subsets of these traits, positive statistical correlations are found (Zera & Harshman, 2001, Houle, 1991). It has also been suggested that negative correlations may not represent true functional trade-offs when there are developmental

or physiological linkages that are independent of adaptation or resources (Agrawal et al., 2010). For example in size-related traits, genes that increase size will also pleiotropically affect a myriad of other physiological properties of an individual (Agrawal et al., 2010). This was evidenced through a study on California Oak, showing that shared environmental variables influencing both reproduction and growth resulted in negative statistical correlations that do not represent the underlying functional trade-off (Knops et al., 2007).

The results from the pathogen exposure experiment show genetic variation in susceptibility to infection with *P. ramosa* though the likelihood of infection was not explained by whether a genotype was long-lived or short-lived. This may be because the cost of resistance is not borne out as a reduction in longevity or it could be because there is no cost at all. Rigby et al (2002) challenge the assumption that resistance must always have a cost, instead suggesting that the likelihood of the cost depends on how resistance is achieved. In *D. magna* this is supported by findings that *D. magna* rarely display resistance costs in comparison to systems in the wider literature (Labbé et al., 2010). It has also been suggested that infections appear less costly in coevolved host-parasite systems like *D. magna* and *P. ramosa* (Hasu et al., 2009, Decaestecker et al., 2007). If a host is from a naïve population investment in resistance is unlikely, so exposure to a parasite would elicit a costly resistance response. In a host from an endemic population that has coevolved with a pathogen, the cost of resistance may appear to be less. A relationship between resistance and longevity may have also gone undetected due to using only one environmental condition. The application of an additional environmental stress like dietary restriction may have revealed costs as has been previously shown in *Drosophila*

melanogaster (McKean et al., 2008), exposing genotype-by-environment interactions.

To conclude, in this series of experiments we show qualitatively similar levels of genetic variation within and between populations in the response to dietary restriction. We also demonstrate extensive within population genetic variation in longevity, however longevity did not covary with reproductive measures or resistnace. An alternative statistical approach, whereby genotypes were allocated to groups by longevity, rather than statistical correlations did not detect a relationship between lifespan and life history measures either. As such, there was no evidence of antagonistic pleiotropy in this system.

Chapter 5 Age and Maternal Age Effects on the Mean and Variance of Pathogen Load

Summary

Super-spreaders are infected individuals that are responsible for a disproportionately high number of pathogen transmission events compared to the population average. Identifying the cause of super-spreaders prior to an outbreak is a longstanding challenge of epidemiological research. Here we studied the crustacean *Daphnia magna* and its bacterial parasite *Pasteuria ramosa* to investigate whether super spreading could be a result of common sources of heterogeneity in a host population – genetic variation, age and maternal age. We examined susceptibility to infection, mean pathogen load and the variance around these mean in pathogen load in young and old hosts and in hosts from young and old mothers. We show that young hosts are more at risk of the super-spreading phenotype as the spore loads were higher and more over-dispersed than in old hosts. There was no effect of maternal age on offspring susceptibility, or the mean and variance of pathogen load.

Introduction

Super-spreaders are individuals that pose an increased risk to surrounding contacts due to becoming infected more quickly, carrying heavier pathogen loads or tolerating infection for longer (Woolhouse et al., 1997). As a result, the distribution of infectious disease aggregates within populations, so that these few individuals are disproportionately responsible for transmission events. However, studies of pathogen dynamics often average individual level performance measures such as contact rates, resistance, tolerance or

pathogen load to describe a whole population (Beldomenico & Begon, 2010, Lloyd-Smith et al., 2005, VanderWaal & Ezenwa, 2016a). For example, the commonly used R_0 , a unitless metric describing the epidemic potential of a pathogen, provides a population average of infectiousness that conceals individual variation (Lloyd-Smith et al., 2005; VanderWaal & Ezenwa 2016). R_0 is a valuable tool to epidemiologists, however, when individual variation is considered in mathematical modelling of infectious disease dynamics, predictions have been shown to change considerably (Lloyd-Smith et al., 2005). Furthermore, studies of past epidemics have shown that super-spreaders have played vital roles in the spread of a wide range of infectious pathogens like the UK foot and mouth outbreak (Woolhouse et al., 2005), vector-borne diseases such as the West Nile Virus (Kilpatrick et al., 2006), zoonotic outbreaks (Mackay & Arden, 2017, Matthews et al., 2013), and sexually transmitted diseases (Stein, 2011). Thus, pinpointing sources of host heterogeneity that could indicate super-spreading capability prior to epidemic outbreak could increase epidemiological predictive and reactive ability.

Heterogeneity in host condition within populations is commonplace and can shape infection dynamics in a number of ways. Host condition can determine an individual's susceptibility or tolerance to pathogen infection and, as the host is the environment the pathogen experiences, can determine how well a pathogen grows within a host. It has therefore been suggested that by considering host condition, super-spreaders may be more easily identified (Beldomenico & Begon, 2010, Tschirren et al., 2007, Tseng & Myers, 2014, VanderWaal & Ezenwa, 2016b). The "vicious circle" hypothesis posits that a host in poor condition, might be more susceptible to infection due to the expense of launching an immune response and may in

turn be exploited more heavily by the parasite, resulting in super-spreading individuals (Beldomenico & Begon, 2010). Resource manipulation experiments on a variety of host-pathogen systems, however, suggest that hosts with more resources (therefore assumed to be in better condition) provide more resources for the parasite, and are less likely to die from parasite induced mortality or other causes (Krist et al., 2004, Seppälä et al., 2008). In addition, there is also greater aggregation of parasite load, amongst hosts in better condition, thus suggesting hosts in better condition are predisposed to super-spreading (Vale et al., 2013). Host age and the age of the parents a host is born to, can determine host condition (Hayward et al., 2015) and have profound effects on resistance, tolerance and epidemiological dynamics within a population (Clark et al., 2017, Drobniak et al., 2014, Hansen et al., 2014, Izhar & Ben-Ami, 2015, Ramsden et al., 2008). It therefore stands to reason that age or parental age could be a source of variation that results in an individual with super-spreading potential.

Furthermore, an individual's pathogen susceptibility may be due to their genotype (Little, 2002). Thus, in outbred populations, teasing apart the sources of individual variation and environmental variation that result in a super-spreading phenotype can be particularly difficult. A solution to this is to investigate pathogen transmission at different ages and in offspring of parents at different ages, in a variety of genotypes, replicated across age groups. We did this using the parthenogenically reproducing crustacean *Daphnia magna* and its natural bacterial pathogen *Pasteuria ramosa*. We conducted two experiments to quantify the effects of genotype, age and parental age on total pathogen load. We then tested for aggregation in the distribution of pathogen load (Vale et al., 2013) as a result of age or parental

age, thus revealing potential reasons why some individuals may or may not be super-spreaders.

Methods

Study System

We conducted two experiments to determine how two common sources of host population heterogeneity, age and maternal age, can affect parasite growth. We used the freshwater crustacean *D. magna*, and its bacterial pathogen *P. ramosa*. *Pasteuria ramosa* infects its host when filter feeding *Daphnia* ingest the spores, which then penetrate the oesophagus, and grow in the host's body cavity until death. When the host dies bacterial spores are released from the decomposing body into the environment where they are ingested by a new host (Ebert et al., 1996, Ebert et al., 2016). Infection is diagnosed by eye as reproduction ceases, and the infected *Daphnia* become red due to bacterial growth in the haemolymph.

Female *D. magna* reproduce parthenogenically, making it possible to produce replicates of the eight chosen genotypes. All genotypes were collected from Kaimes Pond in the south of Scotland (2°20.43'W 55°42.15'N). The same genotypes were used for both experiments. The spores used are a lab strain maintained in a single host genotype from the same population as those used in the experiment.

Experiment 1: Age & Genotypic Variation

Acclimation

Forty-eight replicates of each genotype were acclimatised for three generations under standardised conditions, ensuring independence of

replicates and eliminating unwanted maternal effects. Each *Daphnia* was maintained in a single 60ml glass jar, with 50ml artificial pond medium (ADaM) (Kluttgen et al., 1994) that was changed twice weekly. Jars were kept in trays of mixed ages and genotypes, in an incubator at 20°C, 12hrs:12hrs Light:Dark with each female fed 7×10^6 *Chlorella* spp. algal cells daily. After the third generation two offspring were taken from the second clutch of each acclimatised female to become an experimental offspring. Forty-eight offspring were allocated to be “young” individuals, and 48 to be “old” individuals. No single maternal female contributed more than one offspring for each treatment group. For those allocated to the “young” treatment group, an additional two generations of acclimation were carried out, allowing time for the “old” treatment group to age. This resulted in both age classes being ready for experimentation at the same time.

Experimental generation

“Young” individuals were exposed to 400,000 bacterial spores on their day of birth. “Old” individuals were exposed with the same dose the day they gave birth to their fifth clutch. Spores were counted using a Marienfeld Superior Neubauer Improved Haemocytometer. Exposures were carried out in the glass jars with a teaspoon of sand added to the jar to encourage foraging behaviour and spore contact. After five days the *Daphnia* were changed from the spore solution into fresh water. For “young” individuals their date of birth was recorded, as this was also their date of exposure. For “old” individuals both the date of birth and the date of exposure were recorded. From this point they were maintained as per acclimation until their death. If after 28 days, they were not infected, they were culled. For those who were infected, the date of death was recorded allowing for a record of number of days infected. As parasite transmission spores are released from

the degrading *Daphnia* upon death, all the water and the deceased *Daphnia* were removed from the jar. The contents of the jar were homogenised using a tight-fitting glass homogeniser and centrifuged at 4,200 RPM for 15 minutes. Forty-five ml of spore-free supernatant was removed, leaving 5ml water and spores. This was re-homogenised and then counted using the same protocol as used to measure spore doses.

Analysis

Analysis of proportion infected was carried out using a generalised linear model with whether or not a replicate became infected (yes or no), as a binomial response variable (link = logit) and age (old or young) and clone (8 levels) and their interactions as explanatory variables. To analyse mean spore load, the spore count was standardised by the length of infection period creating a “spores per day” response variable. A Negative Binomial generalised linear model (link = logit) was fit to the data using the MASS package in R v3.5.0 (R Core Team 2017) to test the effects of age (2 level factor) and genotype (7 level factor) on pathogen load as fixed effects.

To test for differences in variance in spore production between age classes, all uninfected hosts were removed from the analysis as they would obviously harbour no spores. First, we calculated the index of dispersion for each age class, as a measure of heterogeneity in the spores counts. This is a ratio of variance to estimated mean describing the length of the tail. At zero there is no variance, at 1, the count fits a Poisson distribution, and above 1 there is aggregation. We then used the *fitdistrplus* package (Marie et al., 2015) to fit negative binomial (NB) and Poisson distributions, first to the whole data set, then separately to old and young subsets of the data. We estimated the mean (μ) and dispersion parameter (κ) of the NB distribution, and lambda

(λ) of the Poisson distribution for each subset of the data using the maximum likelihood estimation method. If the κ parameter is sufficiently large, the NB distribution reduces to a Poisson distribution. We used a likelihood ratio test to determine which distribution fit the data better. We then used a likelihood ratio test, to determine whether the separate estimation of κ and μ for each subset of the data allowed for an overall better fit to the spore count data, than estimating them just once across the whole dataset.

Experiment 2: Maternal Age & Genotypic Variation

Acclimation

Twenty-four female replicates of the same eight genotypes used in experiment 1 were acclimated in an identical manner. After three generations two offspring were taken from the second clutch to become experimental mothers. One offspring was allocated to be a “young” mother, and one to be an “old” mother.

Experimental Generation

“Young” mothers provided offspring from their first clutch, so were put through acclimation generations until the “old” mothers were producing clutch five, analogous to the protocol used in experiment 1 to ensure similar timing for the start of the experiment. From the appropriate clutches one offspring was taken and exposed to *P. ramosa* in the same manner as the previous experiment. Those who did not become infected were culled at day 28. Those that were infected and still alive were culled at 35 days. Those that did not survive to 35 days were not included in the pathogen load counts but were recorded as infected. The infected *Daphnia* that had

been culled at day 35 were placed in 1.5ml Eppendorf tubes with 1ml dH₂O and ground using battery operated grinder. The spores were then counted in the same manner as experiment 1.

Analysis

Proportion infected, and pathogen load were analysed in the same manner as experiment 1 with spores per day as the response variable and genotype (8 level factor) and maternal age (2 level factor) as fixed effects. The distributions were fit to the data as in experiment 1, with the data set containing only infected individuals and split based on maternal age.

Results

Experiment 1.

304 individuals from two age classes and eight genotypes were exposed to *Pasteuria ramosa*. There was a significant genotype by age interaction for the probability of becoming infected (Fig. 5.1A; $\chi^2 = 27.054$, $p = <0.0001$). There were also significant age and genotype effects on pathogen load, however, no significant interaction (Table 5.1 & Fig. 5.2B; $\chi^2 = 28.68$, $p = <0.0001$ and $\chi^2 = 39.53$, $p = <0.0001$ respectively). The index of dispersion was greater than 1 in both age classes suggesting aggregation in spore load counts from both old and young hosts. This was reflected in the better fit of the negative binomial distribution over the Poisson distribution to both the young and old counts (Fig. 5.3A., $\chi^2_1 = 679.74$, $p = < 2.2e-16$ & Fig. 5.3B., $\chi^2_1 = 242.06$, $p = < 2.2e-16$ respectively; Table 5.1), however the index of dispersion was further from unity in the counts from young hosts, suggested there were more overly-dispersed from young hosts than old

(Fig. 5.4). The separate estimation of κ and μ for the young and old subsets of the data significantly improved the fit of the negative binomial distribution to the whole data set in comparison to the single estimation for the whole data set ($\chi^2_2 = 26.098$, $p = < 0.0001$).

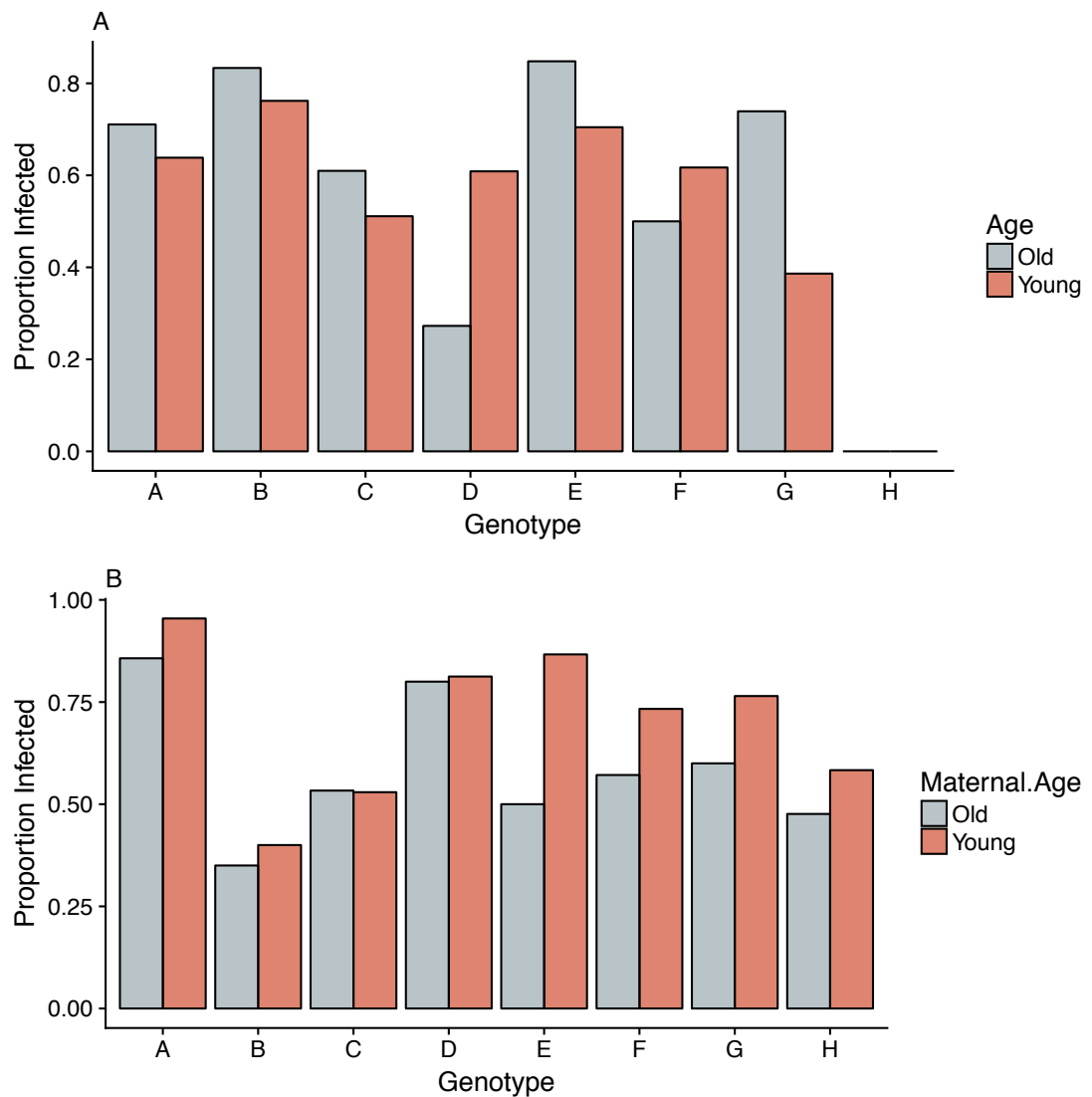


Figure 5.1. A. The proportion of each genotype and age class that became infected when exposed to the bacterial parasite *P. ramosa*. Red represents young hosts and grey represents old hosts.

B. The proportion of offspring of young (red) and old (grey) mothers that were exposed to *P. ramosa* that became infected.

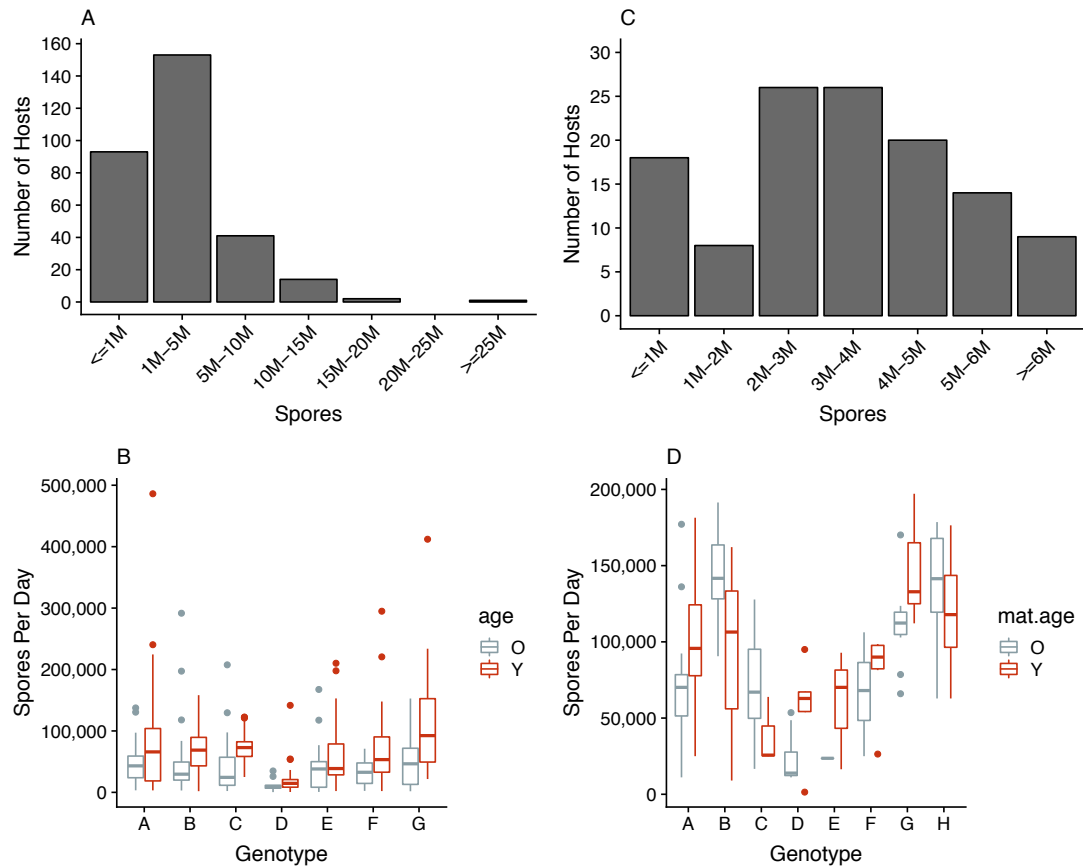


Figure 5.2. A. The distribution of parasite load counts in all hosts, young and old. B. The mean parasite load for in young (red) and old (grey) hosts of each genotype. C. In offspring of old and young mothers, the distribution of parasite load in all hosts. D. The mean number of spores in offspring from young (red) and old (grey) mothers, from each genotype.

Table 5.1. Summary statistics and maximum likelihood estimated parameters from negative binomial and Poisson distributions for both experiment 1 (host age and genotype) and experiment 2 (host maternal age and genotype) showing n – number of replicates. μ - mean spore load $\times 10^4$. S.E μ - standard error of this estimate. σ^2 – the variance. I.O.D – The index of dispersion. κ - the estimated values of negative binomial parameter κ . S.E κ - The standard error of this estimate. LL – the log likelihood value. λ - lambda estimated parameter for the Poisson distribution. S.E λ - standard error of this estimate. ΔLL – the differences between the log likelihood estimates. p – the significance of the difference between the Poisson and NB fits.

	Summary Statistics					Negative Binomial Distribution			Poisson Distribution				
Age	n	μ Spores ($\times 10^4$)	S.E μ	σ^2	Index Of Dispersion	κ	S.E κ	LL	λ	S.E λ	LL	ΔLL	p
Y	155	7.71	0.6	83.58	10.84	1.24	0.16	-479.96	7.71	0.22	-819.8254	339.87	< 0.0001
O	149	4.28	0.35	20.20	4.72	1.35	0.21	-380.03	4.28	0.17	-501.0572	121.03	< 0.0001
Maternal Age													
Y	63	9.97	0.63	21.84	2.19	6.48	2.10	-189.50	9.97	0.41	-204.07	14.57	< 0.0001
O	58	8.97	0.75	26.81	2.99	3.60	1.01	-174.34	9.09	0.4	-201.50	27.16	< 0.0001

Experiment 2.

In this experiment 250 female *Daphnia* were exposed to the pathogen *Pasteuria ramosa*. Host genotype had a significant effect on the probability of becoming infected (Fig. 5.1B; $\chi^2 = 37.31$, $p = < 0.0001$) but there was no effect of maternal age (Fig. 5.1B; $\chi^2 = 3.48$, $p = 0.06$) nor was there an interaction between the two factors (Fig 5.1B; $\chi^2 = 3.44$, $p = 0.84$). There was however a significant interaction between maternal age and host genotype on host pathogen load (Fig. 5.2D; $\chi^2 = 18.732$, $p = 0.0046$). The index of dispersion is higher than 1 for the counts of pathogen load in offspring from both maternal age classes (Table 1). This suggests that the spore counts are aggregated, which is confirmed by the better fit of the negative binomial distribution to the data over the Poisson distribution in both the young and old data subsets (Fig. 5.3C., $\chi^2_1 = 41.786$, $p = < 0.0001$ & Fig. 5.3D., $\chi^2_1 = 54.315$, $p = < 0.0001$ respectively; Table 1). There was no significant improvement in the fit of the negative binomial distribution to the data when estimating κ and μ separately for each maternal age class, suggesting there was no significant difference in aggregation between the two groups (Fig. 5.4B; $\chi^2_1 = -3.4776$, $p = 1$).

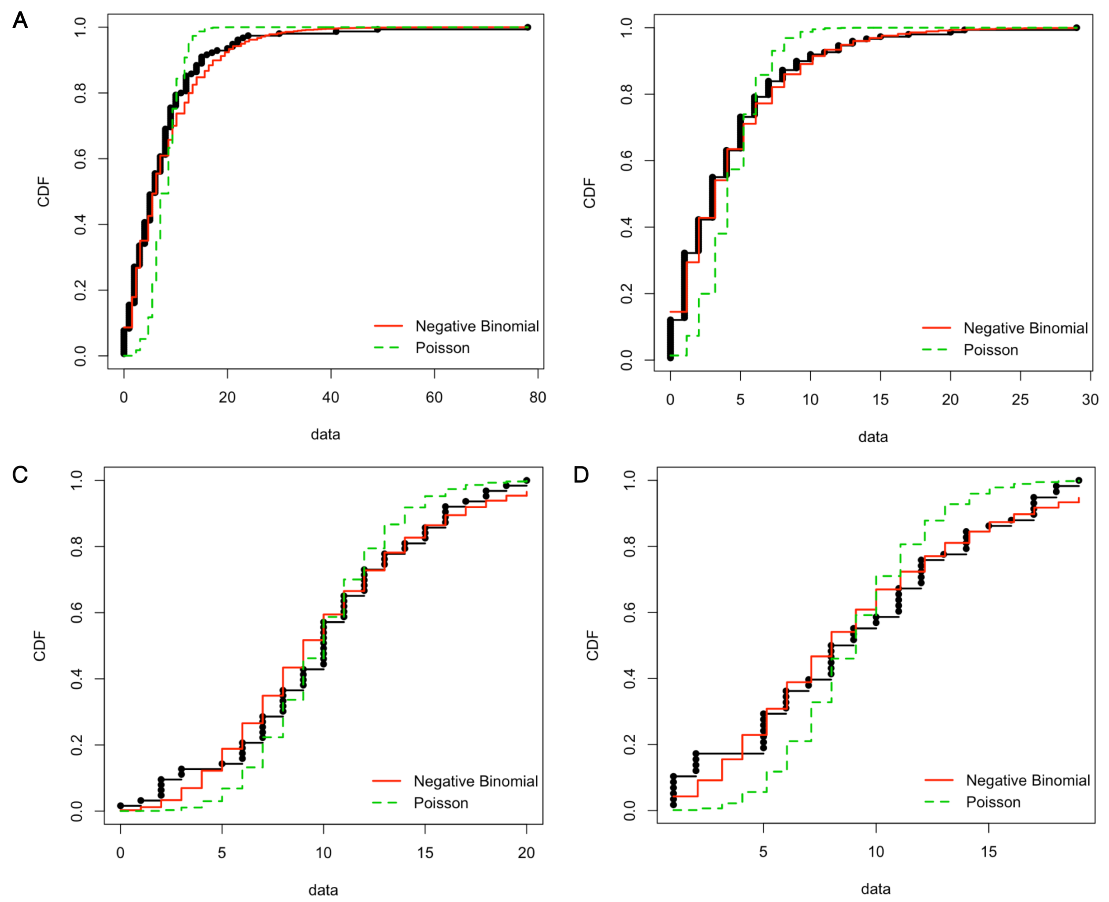


Figure 5.3. A. The cumulative distribution function (CDF) of pathogen load in young hosts, comparing the fit of the Poisson (green) and negative binomial (red) fits to the empirical data (black). B. The CDF of pathogen load in old hosts, comparing the comparing the fit of the Poisson (green) and negative binomial (red) fits to the empirical data (black). C. The CDF of pathogen load in offspring from young mothers, comparing the comparing the fit of the Poisson (green) and negative binomial (red) fits to the empirical data (black). D. The CDF of pathogen load in offspring from old mothers, comparing the comparing the fit of the Poisson (green) and negative binomial (red) fits to the empirical data (black).

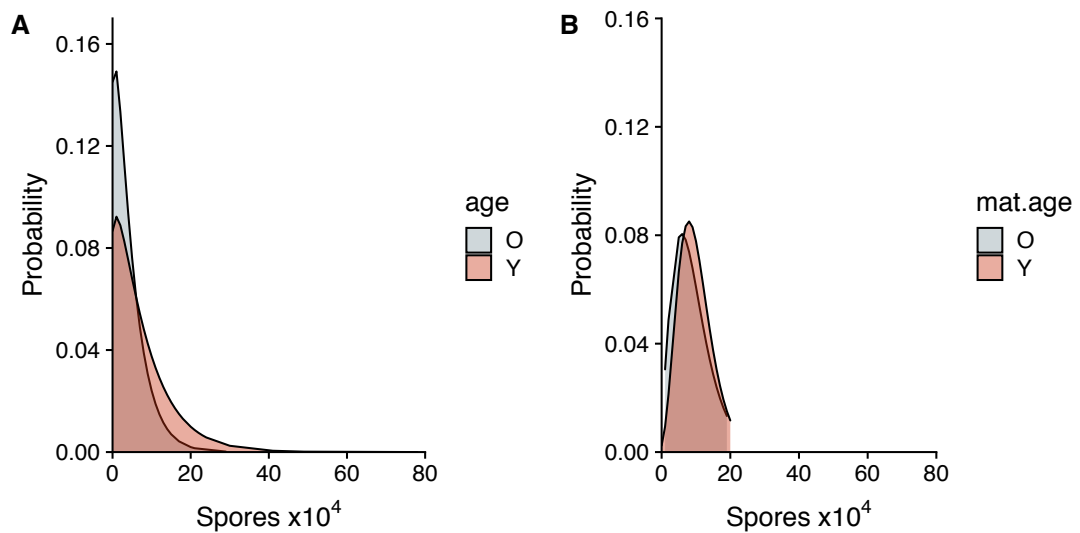


Figure 5.4. A. The estimated probability distribution of pathogen load from young (red) and old (grey) hosts with a negative binomial distribution fit to the data. B. The estimated probability distribution when fitting a negative binomial distribution to pathogen load counts from offspring of young (red) and old (grey) mothers.

Discussion

Understanding what causes an individual to be a super-spreader, and identification of these individuals prior to an outbreak remains a challenge in epidemiological research. In this series of experiments, we sought to determine whether being young or old, or from a young or old mother could affect the distribution (that is to say the aggregation and variance around the mean) of pathogen spore load within each age class. Pathogen loads from young hosts were significantly more over-dispersed when compared to the pathogen loads of old hosts. Young hosts also had a higher mean spore count in all genotypes, and in some genotypes young hosts were more susceptible to infection. These genotypes that are infected when they are young could therefore have longer infection times leading to more transmission events. These results suggest that an individual's age may indicate their super-spreading potential and, in this system, to be young

when exposed to the pathogen would be the highest risk age class for being a super-spreader. There was however, no evidence that maternal age in this system was a source of the super-spreading phenotype, as the distributions of pathogen load in offspring from each maternal age class, were identical.

It has previously been shown that young *Daphnia* are more susceptible to infection than old hosts (Ebert et al., 2016, Garbutt et al., 2014a, Izhar & Ben-Ami, 2015). It is evident now however, that there is considerable genotypic variation in this pattern. *Daphnia* are continuous growers, which confounds age and body size. In both vertebrates and invertebrates, including *D. magna*, larger body size can be indicative of an individual in better condition with larger individuals more resistant to infection (Alto et al., 2008, Coltman et al., 2001, Garbutt & Little, 2017, Vainio et al., 2004). Previously investigated mechanisms for an increase in resistance with size in *D. magna* include filter feeding rate and the timing of moulting. Large *D. magna* have been shown to filter feed faster, and in turn that these faster filtering rates predict a decreased probability of becoming infected with *P. ramosa* (Garbutt & Little, 2017). Other work suggests that the timing of moulting is important in predicting successful infection. The sooner an exposed individual sheds its carapace after exposure to *P. ramosa* the less likely they are to become infected (Duneau & Ebert, 2012a). As young (i.e. small) *D. magna* shed their carapace more often they should in turn be less susceptible to infection (Duneau & Ebert, 2012b, Izhar & Ben-Ami, 2015). In light of the genotypic variation in age-specific susceptibility to infection, susceptibility as a function of body size as explained by these mechanisms, does not hold as some genotypes may be more susceptible to infection

when they are older, therefore larger, whilst others are more susceptible when they are younger and smaller.

Young hosts always produced a higher mean spore load than old hosts. *Pasteuria ramosa* is a castrating parasite, diverting the host allocation of resources from reproduction to growth. (Cressler et al., 2014, Ebert et al., 2004, Ebert et al., 1996, Hall et al., 2007a). It may be that young hosts are allocating more energy to the developing reproductive system, such that *P. ramosa* can in turn acquire more energy than from old hosts with an established reproductive system, resulting in greater pathogen fitness. In addition to having higher mean spore loads, there was more aggregation in the spore counts from young hosts, when compared to those from old hosts. Indeed, some young hosts produced as much as five times the mean number of spores for their specific clone and age. As it was not possible with this data to refit the distributions by age and genotype, it was not possible to determine whether aggregation is associated with a particular genotype. When considered alongside our results showing certain genotypes to be more susceptible to infection when young, and that young hosts produce more spores, it seems plausible that host age could be a cause of the super-spreading phenotype.

There was no evidence to suggest that maternal age was a cause of super-spreading. Maternal effects occur when the environment experienced by a female is reflected in the offspring phenotype. They have been previously linked to a variety of offspring life history and fitness measures (Lynch & Ennis, 1983, Otti & Sadd, 2008, Steiger, 2013) including susceptibility to infection (Boots & Roberts, 2012, Garbutt & Little, 2017, Garbutt et al., 2014b, Mitchell & Read, 2005). When considering the effect of maternal age

on pathogen load distribution however, there was no difference between the two maternal age classes as the distributions of spore load were identical. Considering this in addition to the absence of an effect of maternal age on offspring susceptibility, and the significant maternal age by genotype interaction there is also no evidence to support the commonly held belief that older mothers give birth to offspring in worse condition.

Chapter 6 The Evolution of Virulence in Ageing Populations

Summary

Pathogen replication often causes host damage, limiting the pathogens own fitness. It must therefore balance the need for resources with the need to maintain fitness. Different hosts pose different environments to the pathogen, requiring a shift in this balance. Many sources of host heterogeneity have been shown to influence pathogen virulence evolution, but the role of host age and population age structure has received very little attention. Global populations are ageing at an alarming rate making it prudent to understand how population age structure may affect pathogen virulence evolution. An age-structured optimal virulence model was therefore developed and parameterised with infection data for a selection of infectious diseases of global public interest, and with publicly available demographic data from 2016 and projections for 2050. The model reveals that population baseline mortality, host pathogen susceptibility and host vulnerability to pathogen induced mortality will change as a result of changing population age structure. The resulting changes in pathogen virulence vary by disease, dependent on its sensitivity to changes in each of these components. For example, whilst Tb virulence is predicted to reduce by 4%, Malaria virulence is predicted to increase by 10%. This work also makes obvious a worrisome lack of vital age specific epidemiological data. Furthermore, it makes abundantly clear that biogerontological healthspan research must be extended beyond non-communicable diseases to include immune capabilities and infectious diseases. Contingent on the improvement of age-specific epidemiological data, the model framework presented is suitable for regional and disease specific parameterisation.

Introduction

A pathogen must replicate at sufficient levels to transmit but must balance this with risks to its own fitness. In particular, high pathogen replication may kill the host, shortening the infectious period. This balance between the costs and benefits of host exploitation is the basis of virulence evolution theory, which predicts a pathogen will evolve towards an optimal point of intermediate virulence (Anderson & May, 1982a).

Most populations are inherently heterogeneous, and many of these heterogeneities have been considered in the evolution of pathogen virulence. Interactions between pathogens and heterogeneous hosts have been shown to drive pathogen evolution towards generalist or specialist strategies (Pfennig, 2001, Regoes et al., 2000). It has also been shown that heterogeneities in a population for factors linked to infectious disease dynamics, like susceptibility or tolerance to infection, can exacerbate the effects of co-occurring population structure on virulence evolution (Cousineau & Alizon, 2014). Optimal virulence may also be modified by population contact or spatial structures (Cressler et al., 2016, Herre, 1993, Boots & Meador, 2007) or sex ratio and sex-based heterogeneities in susceptibility or transmission routes (Cousineau & Alizon, 2014, Ubeda & Jansen, 2016). From this it is evident that population structure and heterogeneities between hosts are integral to optimal virulence. However, a common source of population heterogeneity that has gone largely unconsidered is host age.

Hosts of different ages commonly differ in their susceptibility or ability to clear a pathogenic challenge (Garbutt et al., 2014a, Rheins & Karp, 1985, Wilson-Rich et al., 2008, Simon et al., 2015, Izhar & Ben-Ami, 2015). They also differ in other key aspects of epidemiology, like extrinsic and intrinsic rates of mortality. Studied independently, heterogeneity within a population for each of these host characteristics have been shown to be a strong selective factor affecting the optimal virulence in a given population (Alizon, 2008, Yates et al., 2006, Cousineau & Alizon, 2014, Ebert & Mangin, 1997, Choo et al., 2003, Gandon et al., 2007, Mackinnon & Read, 2002), yet they have not been considered in tandem to highlight the overall effects of population age structure on pathogen virulence. This is concerning in light of the unprecedented rate at which human populations are ageing (He et al., 2016). For the first time in human history those aged over 65 outnumber those under the age of five, and between 2015-2030 the proportion of the population aged over 65 is set to increase by 60%. The adaptive ability of pathogens is commonly observed (Davies & Davies, 2010, Brent & Hollomon, 2007, Hemingway et al., 2002), making pathogen evolution in the face of rapid global change of particular interest.

To determine how virulence might evolve in response to ageing populations, a model of optimal virulence was developed considering a trade-off between transmission and pathogen virulence. The transmission- virulence (TV) trade-off suggests that those pathogens producing propagules at a higher rate, transmit more rapidly, but at the expense of killing their host more quickly. The model was parameterised using publicly available demographic and infectious disease data to assess how changes in population age structure will impact the optimal intrinsic pathogen virulence in pathogens of global concern.

The Model

We first lay out the mathematical framework that will later be parameterised with publicly available demographic and epidemiological data. A parasite's basic reproductive rate R_0 links together the numbers of susceptible and infected individuals to determine a pathogen's infectivity and epidemic-potential when introduced to a totally naïve population. If > 1 then the pathogen will spread, if < 1 the pathogen will die out. In its standard form

$$R_0 = \frac{\beta(\alpha)N}{\alpha + \gamma(\alpha) + \delta} \quad 1$$

Where $\beta(\alpha)$ is the transmission coefficient, as a function of pathogen intrinsic virulence (IV), α , which is a trait explicitly of the pathogen pertaining to its fitness. δ is the baseline population mortality rate in the absence of the pathogen, $\gamma(\alpha)$ is the host recovery rate as a function of pathogen IV and N is total host population size. In the presence of a transmission – intrinsic virulence trade-off, as per Frank (1992)

$$\beta(\alpha) = b\alpha^k$$

where b is the transmission value, which in this form describes the rate of successful transmission from an infected host to susceptible hosts as a function of the rate of contact between the infected and susceptible individuals and the susceptibility of uninfected individuals. β increases with

increasing α resulting in a trade-off between host survival and pathogen transmission. The virulence exponent k determines the rate of change in transmission with changing IV (Fig. 6.1; Frank, 1992).

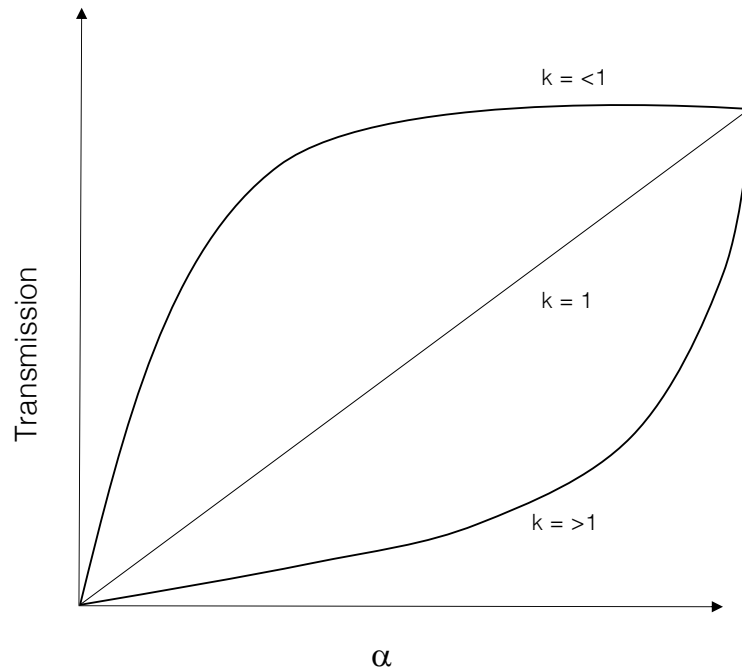


Figure 6.1. . The relationship between transmission and the scaling exponent k , such that when $k = 1$ transmission increases linearly with an increase in intrinsic virulence. When $k < 1$ transmission follows diminishing returns such that continued investment in intrinsic virulence past a threshold yields no further fitness gains to the parasite. When $k > 1$ transmission will rise indefinitely with an increase in intrinsic virulence.

R_0 can also be used to determine aspects of pathogen evolution (Anderson & May, 1982a, Frank, 1992). Here we assume that the realised actual virulence (RAV) of an infection is a function of both the parasite and the host. We therefore adapt Frank (1992), assuming a single infection, but allowing RAV to take the form of αv , where v is the host response (i.e. extrinsic virulence (EV); probability of pathogen induced mortality) such that

$$R_0 = \frac{\alpha^k \beta}{v\alpha + \gamma + \delta} \quad 2$$

Where $\beta(\alpha)$ is as previously.

In this instance we assume no trade-off with recovery. The evolutionarily stable IV rate α^* of the pathogen, that is to say the optimal virulence at which transmission is maximised with respect to IV, is then obtained by solving

$$\left. \frac{\partial R_0}{\partial \alpha} \right| = 0$$

Which yields

$$\alpha^* = \frac{k(\gamma + \delta)}{(1 - k)v} \quad 3$$

By including v , but assuming a single infection, we get a similar expression to Frank (1992). Any changes in virulence can therefore be determined as follows. The present evolutionary stable IV is given by

$$\alpha_o^* = \frac{k(\gamma_o + \delta_o)}{(1 - k)v_o} \quad 4$$

Where o represents original values of age-specific recovery, mortality and EV respectively and

$$\alpha_n^* = \frac{k(\gamma_n + \delta_n)}{(1 - k)v_n} \quad 5$$

gives the new evolutionary stable IV, where n represents new age-specific values of recovery, mortality and EV respectively, then the $\Delta\alpha^* = \alpha_n^* - \alpha_o^*$ so that

$$\Delta\alpha^* = \frac{k(-v_o(\gamma_n + \delta_n) + v_n(\gamma_o + \delta_o))}{(-1 + k)v_n v_o} \quad 6$$

Which is positive when $k < 1$ and $\frac{\gamma_n + \delta_n}{v_n} > \frac{\gamma_o + \delta_o}{v_o}$.

In the presence of population age structure, we assume EV, v , population mortality rate δ , pathogen recovery γ , and the density of susceptible hosts to be a function of host age, so that age specific pathogen R_0^A with a TV trade-off is given by

$$R_0^A(\alpha) = \phi \left(\frac{\beta \alpha^k}{v\alpha + \gamma + \delta} \right) \quad 7$$

where ϕ is N , the density of individuals in each age class, weighted by their susceptibility. Total population R_0 is therefore given by

$$R_0 = \sum_A \phi(R_0^A(\alpha))$$

8

The Data

To determine how changing population age structure will influence pathogen evolution, we used publicly available global infection, and demographic data to parameterise the above framework.

Infectious diseases of public concern were chosen according to the World Health Organisation (WHO) R&D Blueprint Document (2018a) and the WHO World Health Statistics 2018 - Monitoring Health for the Sustainable Development Goals (2018c) lists. From either list, infectious diseases were chosen if relevant data were available from the Global Health Data Exchange (GHDx) database (2018b) so that all data was from the same source. From here we obtained age specific incidence and pathogen induced mortality rate data on the infectious agents of Malaria, Dengue, Ebola, AIDS, Measles and Tuberculosis (Tb). The incidence rate was used to parameterise age specific susceptibility in the model (Fig. 6.2).

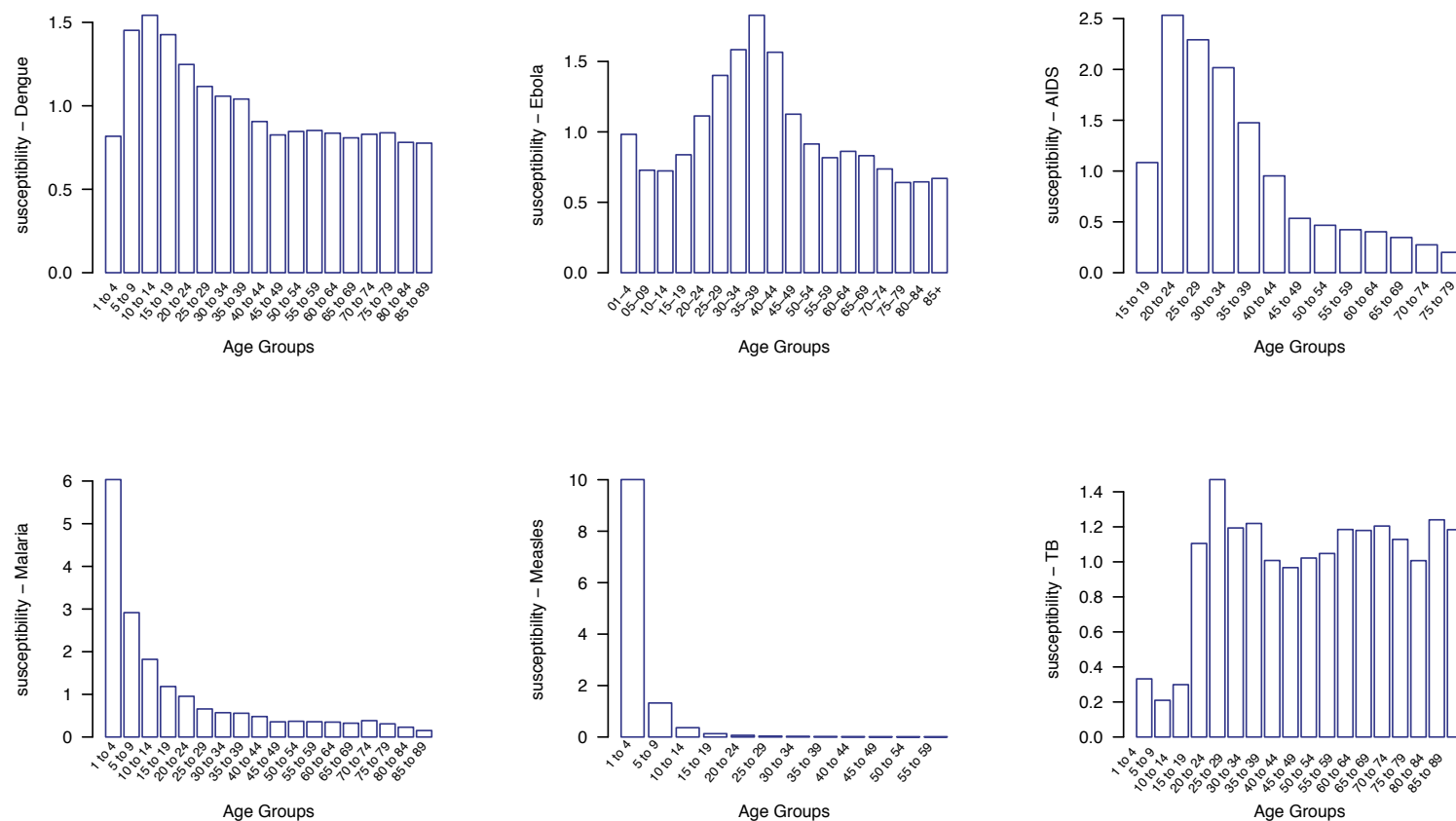


Figure 6.2. Age specific susceptibility to each pathogen determined by age specific incidence rates.

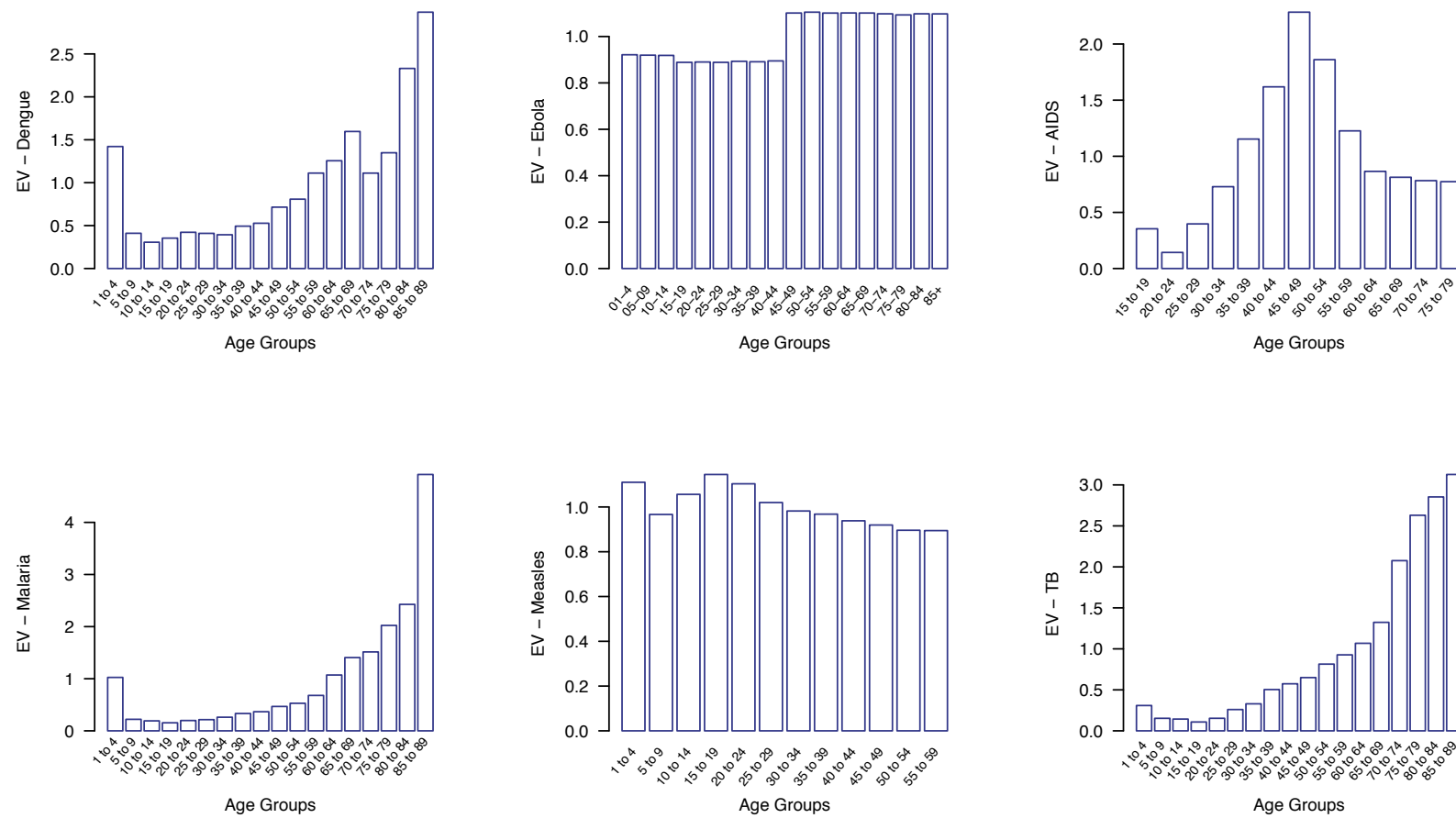


Figure 6.3. Standardised age specific extrinsic virulence, that is the host age-specific vulnerability to mortality for each disease.

Age-specific EV, v , was parameterised as $\frac{\text{mortality}}{\text{incidence}} \text{RAV}$ (Fig. 6.3). Realised Actual Virulence (RAV) was parameterised as the mean pathogen induced mortality across all age classes (i.e. a single value describing the whole population mean virulence).

Age specific population density for 2016 and the 2050 projection (Fig. 6.4A) were obtained from the United States Census Bureau and within the model are used as the proportion of the total population made up by each age class. Global age specific mortality rates (Fig. 6.4B) were provided by WHO. All rates were per 100,00 people for 2016 and were normalised by dividing by the mean across age classes.

Age specific recovery (Fig. 6.4C) was parameterised based on estimates of proportional age specific immune function (Simon et al., 2015). The age specific recovery rate was weighted by 1/ duration for each pathogen to parameterise pathogen and age specific recovery rates. Duration was parameterised as the incubation period plus the symptomatic period. Malaria incubation information was provided by the Centre for Disease Control (CDC) with mean duration of infection from Felger et al (2012). Mean dengue incubation was provided by Chan & Johansson (2012) with the symptomatic fever phase parameterised with estimates for DENV-1 serotype by Nishiura & Halstead (2007). Ebola incubation and duration of infection was parameterised using mean values from Valasquez et al (2015). No mean estimates were available for Measles so the median of the estimate for incubation from the European Centre for Disease Control (ECDC) and the National Health Service (NHS) for duration were used. As latent Tb is non-symptomatic but can develop into transmittable Tb, it is difficult to estimate

the incubation period for Tb. We therefore use only the mean duration with treatment. According to the CDC, Tb is treated for 6-12 months. We therefore use the median of 9 months as duration for infection. We use Monath (2001) to parameterise the mean duration of infection for yellow fever and identical estimates for median incubation periods from Rudolph et al (2014) and Johansson et al (2010). Zika was parameterised with estimates from Lessler et al (2016). Rabies and HIV are assumed to have no recovery.

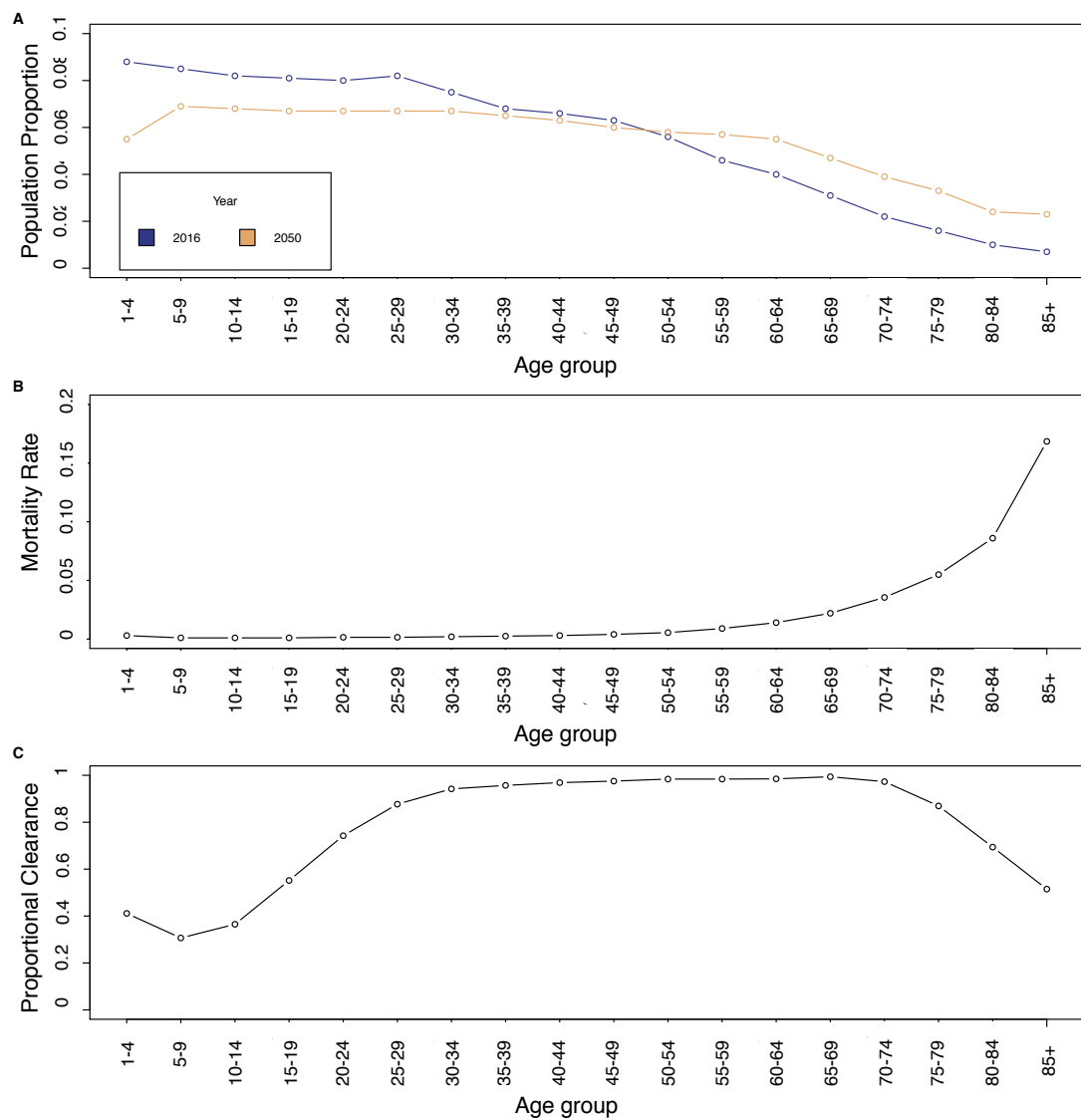


Figure 6.4. A. The Proportion of the population made up by each class for 2016 (blue) and 2050 (orange). B. The rate of mortality for each age class. C. A proportional estimate of clearance capability for each class.

Results

Based on expected changes in age structure from 2016 to 2050, holding all else constant, the mean population mortality rate, δ , is predicted to increase by 81.8% (Fig. 6.5A). Disease independent mean population clearance ability may also increase, though only by 3% (Fig. 6.5B). The slight increase in clearance may be due to the decrease in density of young individuals with undeveloped immune systems at very young ages. Assuming all else remains constant, the population average susceptibility to each pathogen will either remain the same (Tb, Dengue and Ebola) or reduce (AIDS, Measles, and Malaria) in response to the change in population age structure (Fig. 6.5C). The population average recovery rate for each pathogen, however, will remain largely the same (Fig. 6.5D), whilst Extrinsic virulence (Fig. 6.5E) shows disease-specific patterns, with Tb, malaria and dengue rising, and the others either falling or remaining essentially unchanged.

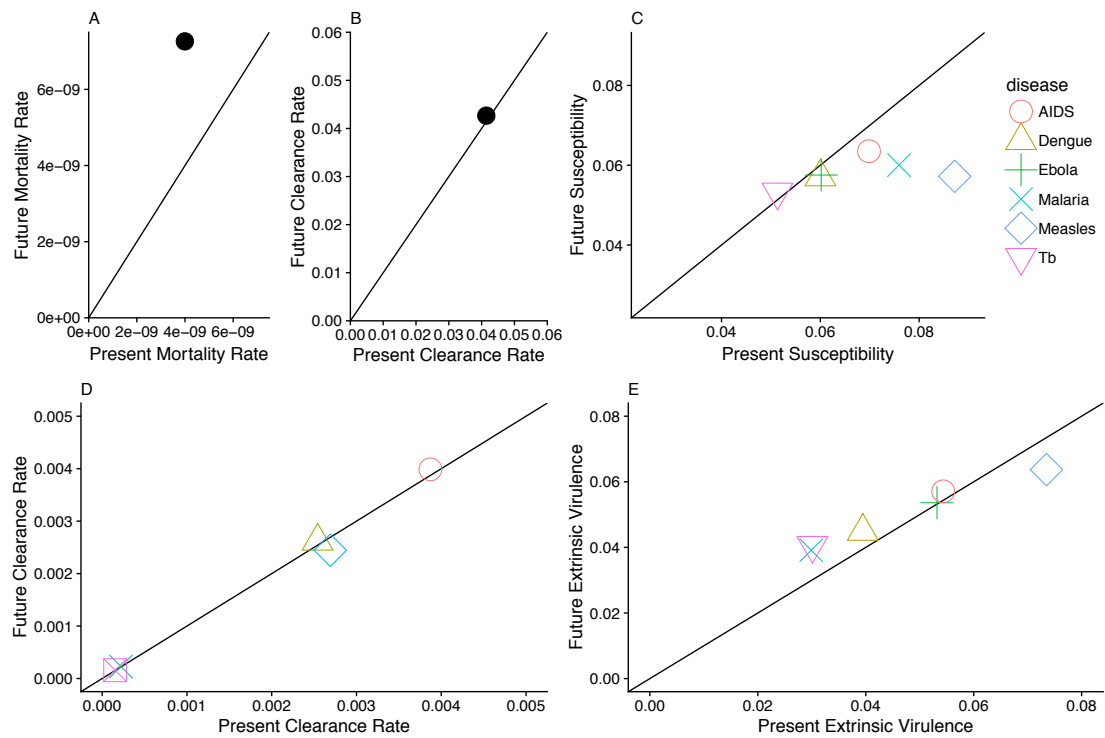


Figure 6.5. Based on changes in population age structure from 2016 (present) and 2050 (future), changes in A. Baseline population average mortality rate B. baseline population average clearance rate C. Population average susceptibility to each pathogen. D. Population average disease specific clearance. E. Population average disease specific extrinsic virulence (host mortality). Above the line indicates an increase and below the line indicates a decrease with changing age structure.

In response to changing age structure, Tb and Dengue are estimated to reduce their intrinsic virulence by 4.02% and 4.23% respectively (Fig. 6.6). These observed patterns can be explained as follows. For Tb, hosts past the age of 20 are the most susceptible to the pathogen, and EV increases considerably with an increase in age (Fig. 6.2 & 6.3). Transmission will increase due to increasing susceptibility without the need to increase IV, which would disproportionately impact old hosts, resulting in an increase in host mortality. Coupled with the increase in baseline population mortality rate (δ ; Fig. 6.5A), an increase in IV would therefore decrease the duration

of infection and thus overall transmission. It is therefore beneficial for Tb to reduce IV in an ageing population. In the case of Dengue, though population susceptibility remains stable (Fig. 6.5C), host vulnerability to pathogen induced mortality increases (Fig. 6.5E) and therefore again this coupled with an increase in baseline mortality rates in the population result in a decrease in IV. Dengue also saturates any gains to transmission with only a small increase in IV (Fig. 6.6 & 6.7) reducing the benefit of, and investment in, IV.

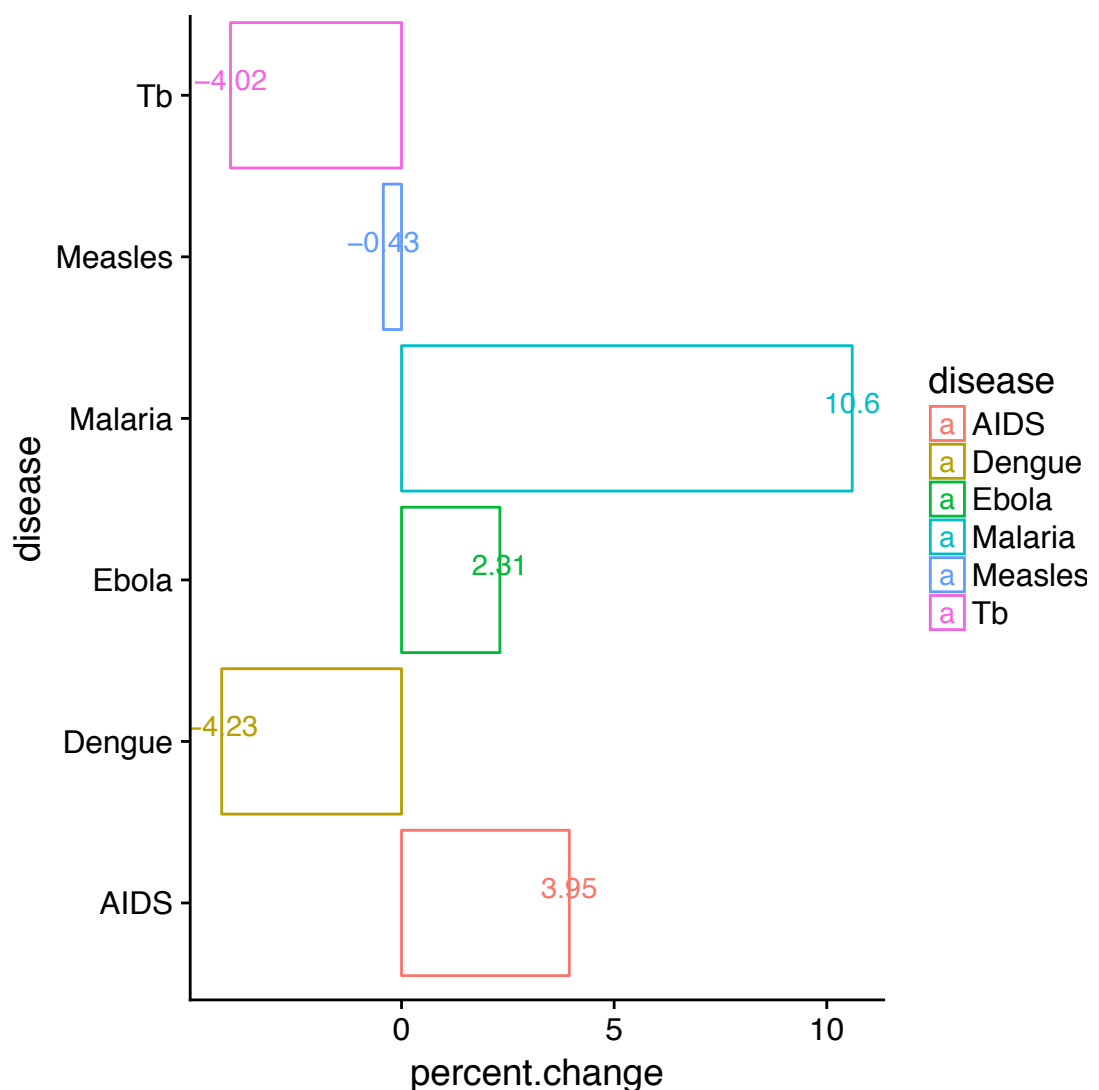


Figure 6.6. The percentage change in intrinsic virulence for each pathogen as a result of changing age structure from 2016 to 2050.

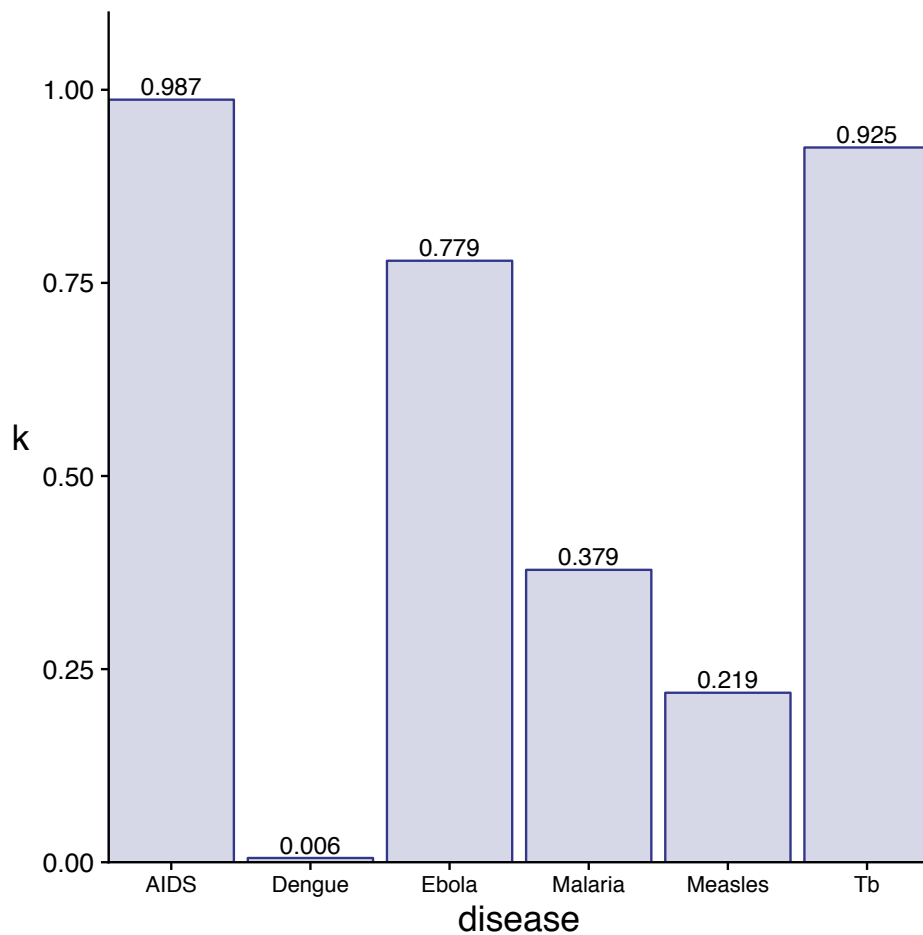


Figure 6.7. The estimated values of k for each of the modelled pathogens

By contrast, Malaria, Ebola and AIDS see increases in their IV with shifts in age structure (Fig. 6.6). Most notably, Malaria increases IV by 10.6%. This striking increase is explained as follows. Population mean susceptibility to Malaria will decrease with an increase in ageing, and therefore less susceptible hosts (Fig. 6.5C). The vulnerability to Malaria induced mortality (EV) will rise slightly (Fig. 6.5E), however, to counter the lowering susceptibility of hosts and maintain transmission, an increase in virulence is necessary. AIDS increases IV by only 3.95% which is likely due

susceptibility, EV and clearance remaining identical with changing age structure, suggesting only changes in baseline mortality are compensated for by changing IV. Furthermore, the estimate of k for AIDS is nearly unity, such that increases in IV result in linear increases in transmission (Fig. 6.8).

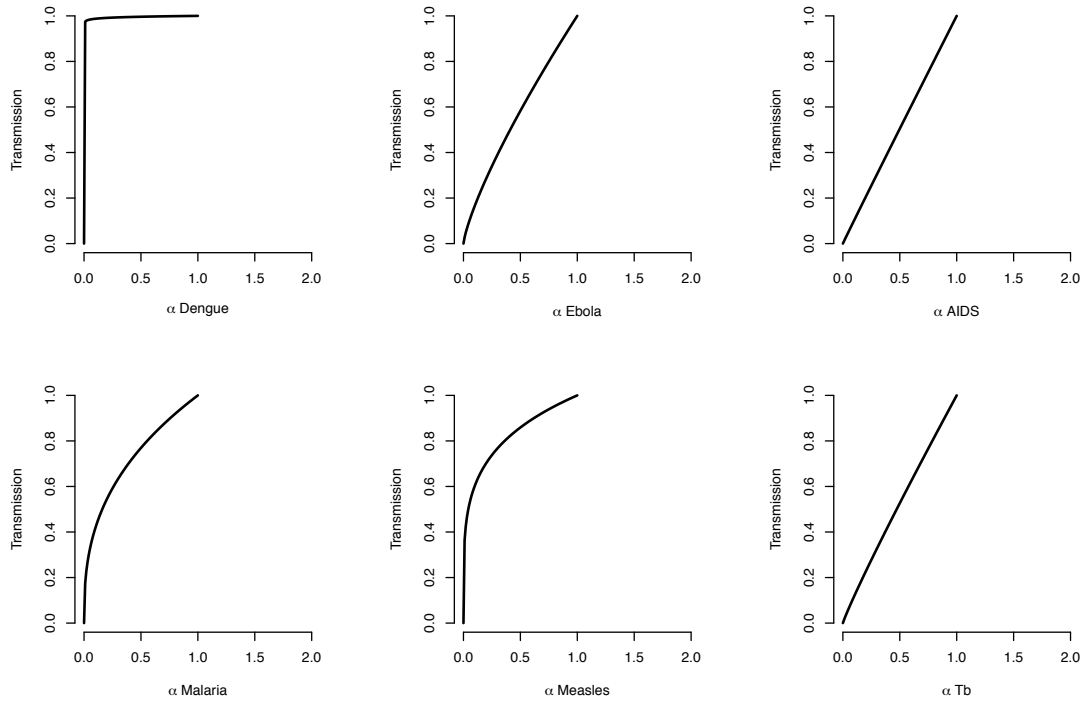


Figure 6.8. The relationship between virulence (α) and transmission is dependent on the virulence exponent k (see figure 5) for each of six pathogens.

Discussion

Here, I show that changing population age structure between 2016 and 2050 could drive pathogen evolution. Six communicable diseases of global interest according to the W.H.O were used to parameterise the age-specific aspects of the model. Shifts in pathogen optimal virulence were disease specific. For example, for malaria virulence is predicted to rise by 10%, whilst for Tb and Dengue it is expected to decline by about 4%. According

to the W.H.O, there are 20,000 deaths a year from Dengue and an estimated 1.3 million Tb deaths. Thus, these reductions in virulence could equate to a reduction of almost 1000 dengue and 50,000 Tb related deaths. On the other hand, the 10.6% increase of Malaria IV would see more pathogen induced deaths. In 2016, there were an estimated 66.1 deaths in children aged 1-4, for every 100,000 people. This increase in virulence would therefore see an increase to 73.1 juvenile deaths per 100,000 people.

Interesting predictions for future demography and epidemiology also emerged. An increase in the rate of baseline population mortality (Fig 6.5A) is expected. When coupled with a decline in fertility, it is indicative of an ageing population (Goldstein, 2009). The decreases in optimal virulence, where increases in the population mortality rate necessitate (i.e. Dengue & Malaria), support previous observation (albeit also with regards to superinfection) that increasing natural host mortality rates decrease the evolutionarily stable pathogen virulence (Gandon et al., 2007). It is also likely that these demographic changes will feedback onto pathogen dynamics, though these were not explored in this model. Of concern is the rising mean probability of host mortality from many infections (Fig. 6.5E), and the maintained mean susceptibilities (Fig. 6.5C) with a shift in age structure. The maintenance of susceptibility to diseases like Dengue, where the incidence rate is currently aggregated in young hosts, suggests that the large increase in ageing individuals is sufficient to maintain population susceptibility to the pathogen.

The increasing probability of mortality for many of the pathogens, as the global population ages, ultimately suggests efforts may be best spent preventing the degeneration of immune capability, rather than being left

responding to it. Patterns such as these draw attention to the need for a focus on healthspan rather than lifespan from both members of both biogerontological and epidemiological research. As it stands however, healthspan is largely considered in the context of non-communicable diseases, which are now commonplace as they develop over increased lifespans (Crimmins, 2015).

The trade-off between transmission and intrinsic virulence, which in this model was described by the virulence exponent k , though often referred to as the archetypal trade-off a pathogen faces (Alizon, 2008, Alizon et al., 2009), to the best of our knowledge has never before been numerically estimated. There is an opportunity for this framework to be applied to more pathogens, and for these estimates to be validated experimentally. In addition, the application of this framework to pathogens that elude control despite exhaustive efforts, could provide great insight into the limitations of our interventions and new ways to limit pathogen growth based on pathogen life history optimization (Ross et al., 2014). This does, however, require high quality data. Indeed, the accurate parameterisation of this model was greatly hindered by a lack of age-specific epidemiological data. For example, ideally, clearance would have been parameterised as an age specific rate of clearance for each pathogen. This requires data on the age at infection, and continuous monitoring and recording of pathogen load for the duration of the infection. This data was not available for most pathogens, however, and in the rare instances when it was, resolution was low. There may be limiting factors to the collection of this high-resolution data. For instance, the infectious rhythms seen in Malaria infections as a result of parasite replication cycles (Mideo et al., 2013) may impede collection. Alternatively, the lack of hospitalisation due to illness, which would provide a

platform for such data collection, may not be possible. In any case, clearance was therefore parameterised as $1/\text{duration of infection}$ weighted by an approximated age-specific proportional clearance ability obtained from Simon et al (2015).

This approximation of clearance was also very general, due to a lack of quantitative data on age-specific immune capability. This lack of quantitative data on age-specific immune function is concerning, when changes in the immune system are so commonly cited as the reason for reduced resistance to infection at old age. The mechanistic parts of human immune systems have been characterised throughout life (Montecino-Rodriguez et al., 2013, Hasselquist & Nilsson, 2009, Simon et al., 2015), though it would appear that these reductionist, mechanistic findings must now be brought together to quantitatively describe whole biological systems. Such data would be invaluable to epidemiological research.

The assumptions that there would be no other demographic or epidemiological changes to parameters such as baseline mortality rate, incidence rates, or changes in pathogen induced mortality rates occurring between 2016 and 2050 were suitable for the simplification of the model. Indeed, rates of mortality have recently plateaued in many countries, bucking the trend of globally reducing rates of mortality seen in previous years (Dicker et al., 2018). However, the use of global rates and global population age structure to numerically parameterise the model overlooks important ecological differences in pathogen distribution. For example, according to the W.H.O, Nigeria had the highest number of Malaria caused deaths in 2017. The population age structure of Nigeria in 2017 sees almost double the number of young infants, and only a third of the number of

individuals aged over 65, in comparison to the global proportions used to parameterise the model (Appendix B; Fig. 9.1). Importantly, however, this framework can be easily re-parameterised with regionally specific demographic and infectious disease data.

In conclusion, this model suggests that shifts in population age structure will impact demographic and epidemiological parameters that could impact the evolution of pathogen virulence. The classic transmission – virulence trade-off was numerically parameterised for the first time and highlights the idiosyncrasies of the trade-off in the ecology of each pathogen. This work highlights the need for healthspan to be considered in the context of infectious disease, as well as the need for more detailed age-specific epidemiological data.

Chapter 7 General Discussion

Throughout this thesis I show that age, the process of ageing and population age structure have significant effects on pathogen dynamics beyond the common consideration of contact rates. In doing this, I also characterise ageing across a suite of traits connected to epidemiology.

In Chapter 2, I show that old mothers give birth to larger, more resistant offspring. Considered alongside previous observations of improved resistance with an increase in age in the *Daphnia magna* - *Pasteuria ramosa* system, I then show that the long-held dogma that increasing extrinsic mortality results in decreasing transmission does not hold true when considering age and maternal age-specific differences in susceptibility. In this case transmission initially increases with an increase in extrinsic mortality.

In Chapter 3, my experimental results challenge long-standing ageing theory, showing that ageing does not always result in a decline in physiological capability, and that ageing across traits within an individual is not synchronised. By using epidemiological models, I then show that ageing impacts pathogen transmission in two ways. First, ageing can affect pathogen transmission directly, through effects on individual susceptibility and maternal age effects on susceptibility. Secondly, ageing can influence pathogen dynamics indirectly, through effects on demography – namely reproduction rates and mortality rates. Using this framework, I also show how these effects of age on transmission are ecologically sensitive, and that they interact to produce dynamic transmission landscapes. Lastly, I show that the manipulation of senescence in humans across traits may increase

pathogen transmission if ageing is not considered as a suite of traits that interact.

In Chapter 4, I conduct a series of experiments to explore genetic variation in longevity and life history trade-offs. I show that there is a comparable level of genetic variation in longevity within a population, as to between populations, challenging common expectations. I then show that there is no evidence for the maintenance of this genetic variation through antagonistic pleiotropy, as there were no significant relationships between reproduction, pathogen resistance and longevity. These relationships were explored using an alternative experimental and statistical approach with more power, but which yielded the same answers, confirming that there is no detectable statistical relationship between the life-history traits.

In Chapter 5, I show that host age may be a contributing factor to the super-spreading phenotype. Mean pathogen load was higher in young hosts of all genotypes and was significantly more over-dispersed in young hosts. Maternal age however, was not a source of the super-spreading phenotype.

In Chapter 6, I turned my attention to pathogen evolution, developing an age structured model of pathogen optimal virulence. The model was parameterised with publicly available data for a collection of pathogens of public interest according to the WHO. I show that population age structure impacts all aspect of pathogen R_0 and that the changes to population age structure from 2016 to projections for 2050 are enough to drive changes in pathogen optimal virulence.

Each data chapter of this thesis concludes with a focused discussion. This chapter will therefore discuss overarching themes relevant across chapters, and the broader implications of this research.

Daphnia magna as a Model Organism in Ageing Research

Previous work has exhaustively studied the ecology and evolution of *D. magna* and the dynamics of *P. ramosa* infections (Ebert, 2005, Ebert et al., 2016 and citations within). Within this however, age and maternal age effects on individual performance have until now received very little attention (Garbutt et al., 2014a, Izhar & Ben-Ami, 2015, Izhar et al., 2015). In ageing research more generally, *D. magna* is far less commonly used as a model system for ageing when compared to the likes of the nematode *Caenorhabditis elegans*, the dipteran *Drosophila melanogaster*, or mammalian model organisms such as mice and primates (Partridge et al., 2018). The work presented in this thesis, however, highlights the suitability of *D. magna* to ageing research, largely due to their experimental replicability, tractable life history and manageable lifespan, but also due to their clear plastic responses to environmental manipulation.

There is also evidence to support the role of *Daphnia* as a model organism in mechanistic ageing research. Perhaps one of the most conserved and well-studied pathways in ageing, the Insulin Signalling Pathway, has been extensively characterised across taxa (Sen et al., 2016). Though more commonly investigated in mice and *Drosophila*, there is evidence that *Daphnia* would make a suitable model organism for studying this pathway, as similar Insulin Signalling Pathway characteristics to mammals were detected (Boucher et al., 2010). Another example exemplifying *Daphnia* as a suitable model of mechanistic ageing research was the discovery of Sir2

activity in *Daphnia*. Sir2 plays a vital role in the repair of molecular damage, especially within the context of ageing (Schumpert et al., 2016).

Furthermore, one of the main reasons for change in gene expression with age is epigenetic regulation (Moskalev et al., 2014). Epigenetic ageing hypotheses suggest that maladaptive epigenetic alterations are fundamental to ageing. MicroRNAs (miRNA) manage and fine tune the ageing process (Moskalev et al., 2014) where the dysregulation is associated with age-related diseases like cancer (Leung & Sharp, 2010). In *Daphnia*, miRNAs expression patterns in response to dietary restriction were similar to other organisms where miRNAs are a key regulator of the downstream effects of dietary restriction (i.e. lifespan extension and apoptosis) (Hearn et al., 2018).

There has more recently been a call in ageing research for the integration of reductionist mechanistic research, where some see ageing as only a single mechanism such as telomere attrition or free radical damage, with more complex whole system interactions, to more precisely characterise ageing (Barzilai et al., 2012, Kirkwood, 2008b, Kirkwood, 2008a). The evidence that *D. magna* is suitable for both observational and mechanistic approaches to ageing research suggests that *D. magna* is a valuable tool in ageing research in an age of systems biology.

Modelling Benefits and Limitations

When building a suitable model, there will be a trade-off between simple and strategic, with the direction of the trade-off decided by the purpose of the model. It has been proposed that despite the type of model chosen, all models can be used as tools in three ways; 1. To simplify the real world. 2. Rather than corroborating causal hypotheses, they limit the search space of

competing hypotheses and 3. Theories and models can be used to inform empirical works (Peck, 2004 though see Cooper, 2003). Deterministic models, as used in this thesis, are a powerful tool to understand the dynamic behaviours of systems. They are tractable and interpretable and can therefore often be solved analytically, informing understanding on how certain biological processes influence one another. They therefore aid the development of basic theory. They are however, based on the hypothesis that population sizes are large, and can therefore be described by averages (Lachor et al., 2011). This often means they are highly simplified versions of the system of interest, only accurately describing the most simple of biological systems (Lachor et al., 2011). For the purpose of the work in Chapters 2 & 3, they were suitable approximations of the systems of interest, allowing the link between age-specific performance and disease transmission to be investigated, and indeed to inform further investigation. In the context of public health and predictions however, quantitative simulations are necessary. These simulations are far more capable of handling biological complexities including random events and chance, and it has been suggested should be considered as a platform for experimentation when logistics, ethics or budgets constrain possibilities (Peck, 2004).

A major limitation to simulations was touched upon in Chapter 6 where the estimates were dependent on parameterisation with accurate data, which as it stands do not exist. Simulations are therefore limited by what is available. They are also limited by their own complexity; when systems become so multifaceted that the model cannot be interpreted, the strategic benefit is nullified (Brauer, 2017). The limitations to stochastic and simulation models as a result of lacking data availability highlights the importance of the

systems biology approach and harks back to Cooper's (2003) three ways in which modelling can be used as a tool. Observational data is produced, which informs the development of analytical models. These models inform theoretical hypotheses that enable further data collection and the parameterisation of more complex models and simulations. These in turn also produce hypotheses and therefore inform empirical investigations.

Juvenile Susceptibility

In Chapter 6, the incidence rate (parameterising susceptibility in the model) for many of the infectious agents aggregates in young hosts. In Chapter 5, young hosts are also sometimes more susceptible to infection, but always produce higher pathogen loads with greater variance. Physiological arguments are often given to explain the presence of juvenile susceptibility, or improved adult resistance where one might expect immuno-senescence, for example, under-developed acquired immunity or factors connected to passive resistance like undeveloped exoskeletons or moulting (Duneau & Ebert, 2012a). However, given that fitness costs are highest when young, and infection can limit fitness through early mortality or limitations to reproduction, particularly in the case of sterilising pathogens, one would expect that selection would favour juvenile resistance.

One theory for the development of juvenile resistance is that immune responses may be costly. The presence of age-specific resistance polymorphisms in a population would support this theory. If there was no cost to resistance, then the presence of an endemic disease (like *P. ramosa* in natural *D. magna* populations) would increase the frequency of juvenile resistance (or more precisely the resistance allele). However, as shown in Chapter 5, there was genetic variation for age-specific susceptibility. Costs

could be evidenced by the extension of lifespan when down-regulating the immune system (Lee et al., 1999, DeVeale et al., 2004, Pletcher et al., 2002, Seroude et al., 2002), or when negative relationships between reproduction and immune function are observed (Sheldon & Verhulst, 1996). Though the results in Chapter 4 do not support this hypothesis as there was no relationship between longevity and resistance. As discussed in Chapter 4 however, the lack of trade-off may not reflect the underlying functional relationship. Furthermore, mathematical investigations into the maintenance of genetic variation for resistance have revealed the potential existence of highly complex trade-offs and feedbacks (Antonovics & Thrall, 1994, Boots & Haraguchi, 1999) that limit the maximisation of the resistance phenotype. These costs and trade-offs in context of the evolution of juvenile resistance have only more recently been theoretically explored by Ashby & Bruns (2018). In the context of *Daphnia magna* and *Pasteuria ramosa*, the Ashby & Bruns models shows that when juvenile susceptibility is traded off against adult fecundity, selection for juvenile susceptibility is strongest when lifespan is short or long and the probability of reaching the adult stage is low. This would apply to *Daphnia* and could explain the maintenance of juvenile susceptibility in their populations.

Transgenerational Ageing

In Chapters 2, 3 and 5 the transgenerational effect of age on offspring performance was considered. In Chapters 2 and 3 improved performance was seen in offspring of old mothers, and in Chapter 5, maternal age did not contribute to the super-spreading phenotype. These observations challenge medical and ageing theory dogma that old mothers produce poor offspring as reproductive performance allegedly declines with age. Alternatively, these data show that reproductive ageing should be considered

transgenerationally, so that offspring condition, coupled with offspring performance is used as a metric for age specific reproductive performance. The quantification of offspring investment has been addressed a number of times but is generally confined to measures of offspring number vs. mass, or is often measured in roundabout ways like ovary mass or follicle/ unlaidd egg counts post mortem (Smith & Fretwell, 1974, Camargo et al., 2008, Cassai & Prevedelli, 1998). From the data presented in this thesis however, it is clear that a more direct measure of investment would be to consider offspring performance. In the context of evolutionary ageing theory, where fecundity represents fitness gains, an old female producing fewer offspring may not experience lower fitness if those offspring have the same reproductive and survival success as those from younger mothers (Chapter 3 Appendix), in addition to increased resistance to infection as was seen in Chapters 2, 3, and 5. In Chapter 4, no trade-offs were detected between longevity and reproductive performance or the rate of senescence as only fecundity was measured. More may therefore be gained when looking for trade-offs by considering transgenerational reproductive effort over simply fecundity. This does raise the more philosophical question of who the phenotype then belongs to and who the fitness benefit is then attributed to (Wilson et al., 2005), although as stated in the title of Wilson et al (2005), does it really matter?

Healthspan over Lifespan

The doubling of lifespan between 1900 - 2010 was initially due to focussed efforts to reduce pathogen induced and infant mortality (Crimmins, 2015), with more recent gains attributed to factors like improved nutrition, water, hygiene and overall living standards (Crimmins, 2015, Partridge et al., 2018). Though the improvement in expected lifespan is often also attributed

to the improvement in medicine, the extent of its impact may be over-estimated as it has been shown that the improvement of childhood physical condition and childhood survival is sufficient to explain longer life in adulthood (Crimmins, 2015 though also see; , Beltran-Sancheza et al., 2012, Finch et al., 2014). This is an important point to make, as infant mortality globally has been considerably reduced (as shown in Chapter 6 Fig. 6.4B where global infant mortality is approaching 0). This may suggest we are approaching a limit of possible life extension, if the assertion that childhood condition and mortality determines longevity by Crimmins and colleagues is true (though, this does go against a wealth of evidence to suggest that there are many mechanisms that could be exploited to extend lifespan (Flatt & Partridge, 2018, Partridge et al., 2018)). The increase in lifespan is important when considering how little has been done until more recently to compress the associated period of morbidity (Crimmins, 2015). When lifespan was short, individuals died of acute infections. Now however, it is more common for people to die from chronic infections that develop over the increased lifespan (Crimmins, 2015). Because of this, medicine is now focused at later ages meaning more people are living with disease and disability for longer.

Though much of the work in the area of healthspan vs. lifespan is focused on non-communicable diseases like cancer, cardiovascular and neurodegenerative diseases (Partridge et al., 2018), in Chapters 3 & 6 I show that it is also important in the context of pathogen transmission and the impact of healthspan at the population level. In Chapter 3 I show that the extension of lifespan, without an improvement in resistance to infection could drive an increase in pathogen R_0 . This is in keeping with the simulations of non-communicable disease by Crimmins et al (1994), who

show that if both mortality and morbidity are improved, then the proportion of lifespan spent in good condition is maintained, whilst the population of unhealthy individuals can decrease. When mitigating only mortality however, overall population health declines. In Chapter 6 the importance of healthspan is also highlighted in many of the pathogens by the maintained mean population susceptibility to some infections with changing age structure, despite a decrease in highly susceptible young individuals (such as is seen in Dengue) and the increase in population mean pathogen induced mortality due to the increase in ageing individuals. Rather than considering disease and age specific treatments however, it has been suggested that a more advantageous approach would be to focus on healthy host ageing to reduce susceptibility and immuno-senescence, rather than to focus on the complex ecology and age specific infectiousness of each pathogen (Kaeberlaein et al., 2015).

Concluding Remarks

In this thesis, I have shown the age-specific and maternal age effects on resistance and susceptibility can increase pathogen transmission in unexpected ways. Further to this I have I have shown that the consideration of only mortality and fecundity in ageing research is limiting and that a more complete picture of ageing would be gained from considering transgenerational effects of age on individual performance. Furthermore, there is considerable genetic variation in the life history traits found to be connected to pathogen transmission. I also show that pathogen optimal virulence is a function of population age structure, such that ageing of global populations may drive changes in pathogen optimal virulence in a range of diseases of public health interest. These results draw attention to a previously unconsidered aspect of population structure that impacts

pathogen transmission, and illuminate an unmistakable link between host age, maternal age, population age structure and pathogen dynamics.

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Chapter 8 Appendix A. Chapter 2

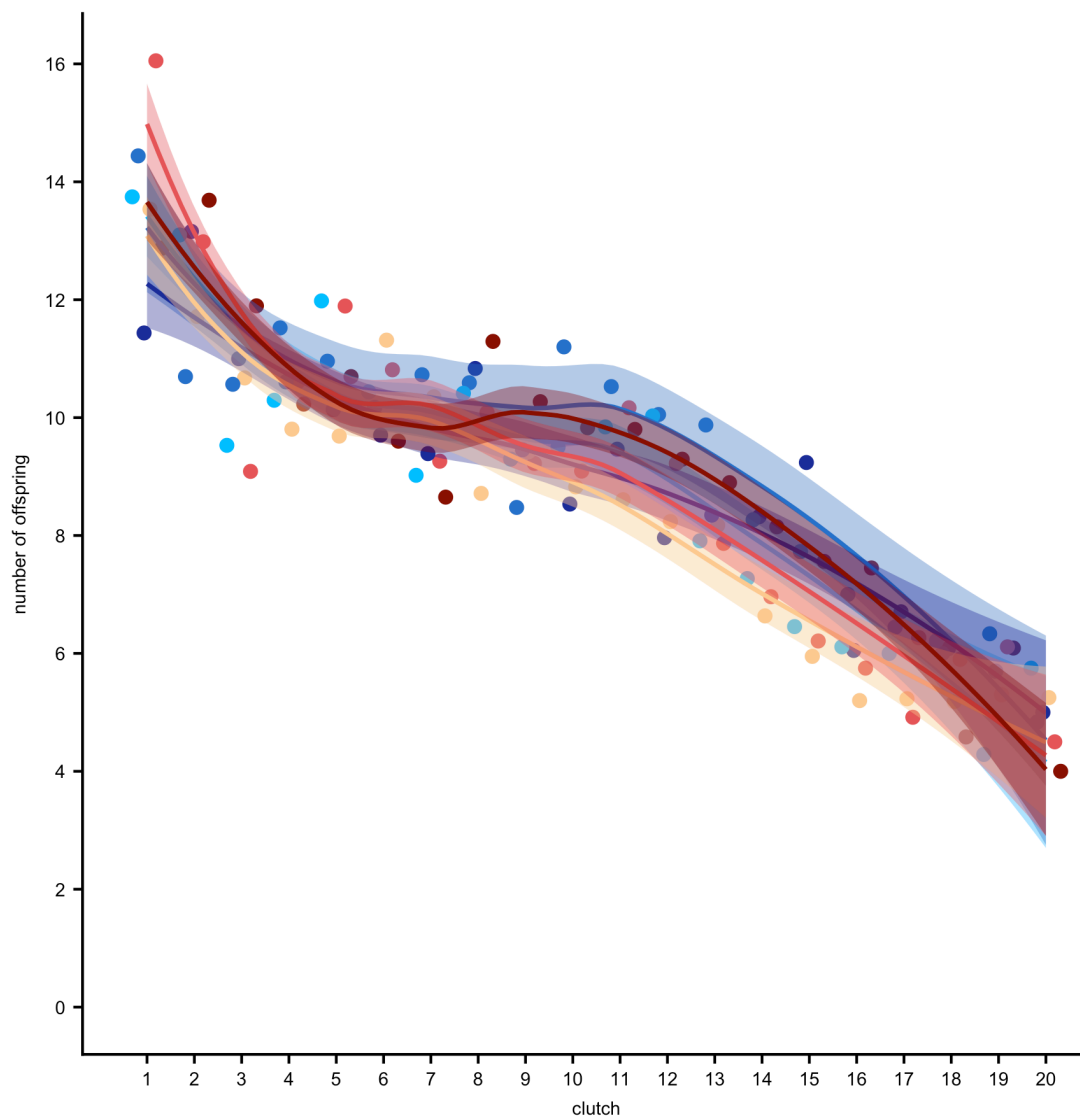


Figure 8.1. Number of offspring produced at each reproductive event plus standard error shading, from clutches 1-20, in offspring from primiparous (A), multiparous (B) or grand multiparous (C) mothers on caloric restriction (LF; blues) or normal food (NF; reds) levels where maternal age had no effect, and maternal caloric restriction mildly reduced rates of reproductive senescence. Shading surrounding lines represents standard errors

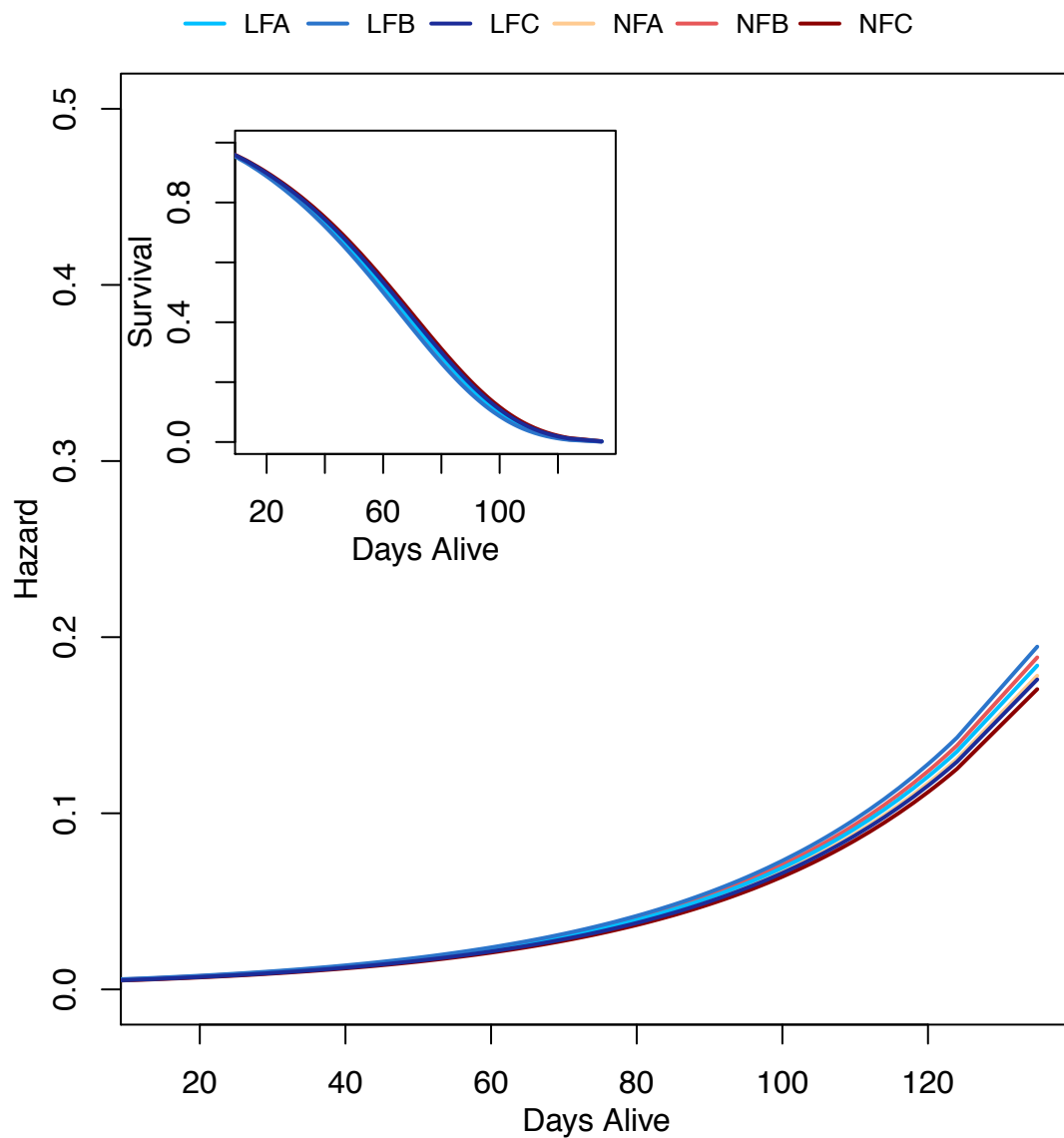


Figure 8.2. The survival and hazards of offspring from primiparous (A), multiparous (B) or grand multiparous (C) mothers on caloric restriction (LF) or normal food (NF) where there is no significant difference in survival or hazards, between groups.

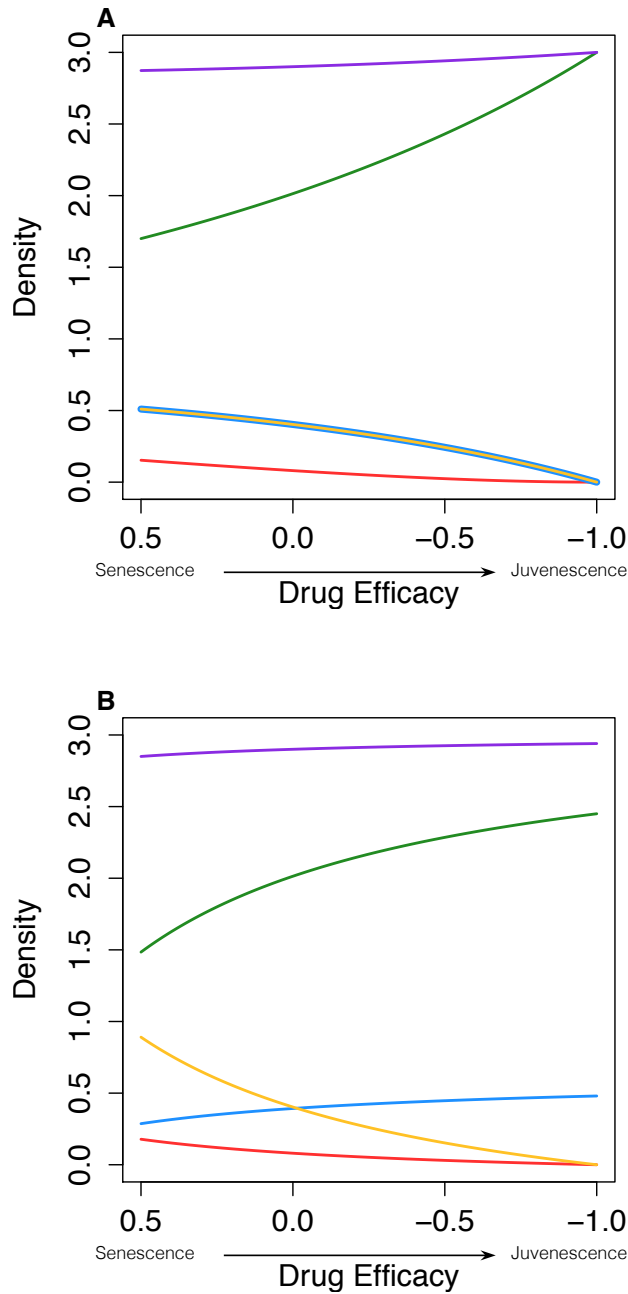


Figure 8.3. The population density and age structure as ageing is ameliorated in humans. with respect to A. a reduction in mortality senescence and B. A reduction in reproductive senescence. The purple line represents total population density (N_u), whilst red is young individuals from young mothers ($U_{Y,Y}$); blue is young individuals from old mothers ($U_{Y,O}$); yellow is old individuals from young mothers ($U_{O,Y}$); green is old individuals from old mothers ($U_{O,O}$). Other parameters are $\delta = 0.1$ (low mortality); $r = k = 3$; $m = 0.5$; $\alpha = 1.5$; $\beta = 3.5$

Chapter 9 Appendix B. Chapter 6

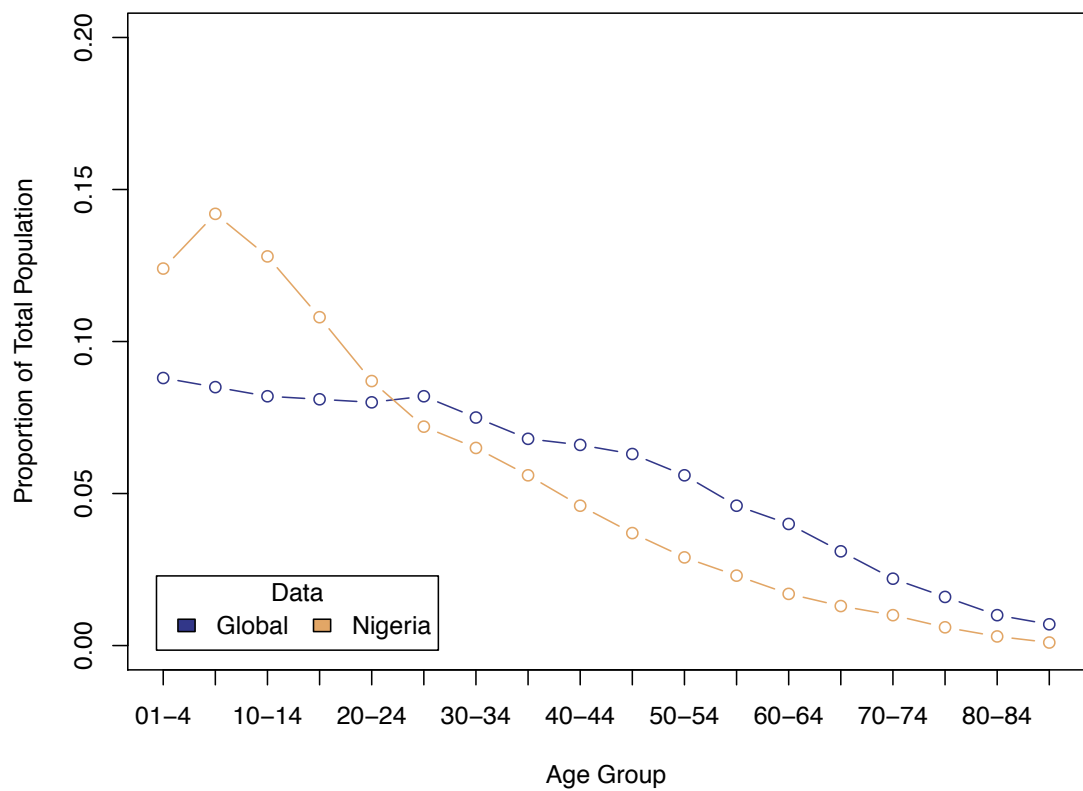


Figure 9.1. Population age structure for global values (2016) and Nigeria (2017).